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ANNOTATIONS, MINOR CONTRIBUTIONS, QUERIES

Under the above caption will be published from time to time comments, criticisms and suggestions on technical procedures; minor contributions such as laboratory aids and short cuts which are not considered sufficiently important to warrant a formal paper; and queries.

Obviously comments and criticisms should be signed; queries should be signed but names will be withheld on request; full credit will be given those who contribute laboratory aids, short cuts and the like.

An attempt will be made to obtain answers from authoritative sources to the queries submitted. It must be emphasized that the views expressed in this department are not the opinions of any official body.

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BACTERIAL FILTERS AND FILTRATION TECHNICS*

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Filters capable of withholding bacteria are essential items in every bacteriological laboratory. Their use for the sterilization of certain culture media, toxins, sera, antigens and many other heat labile substances, the preparation of bacteriophage, the isolation and study of viruses and in the study of bacterial life cycles is an absolute necessity. The selection of the proper filter is no small problem for the laboratory worker and an even greater task is the acquisition of a satisfactory technic for the manipulation of each particular type of filter. Nowhere in the literature is there assembled a list of filters now available to the worker and a suggested practical technic for the assembling and use of each filter. It is the purpose of this paper to present such practical material for which there has long been need.

In 1928, Mudd[23] described the types of filters then available as well as presenting the physical chemistry and a historical treatise of bacterial filtration. Such material will not be repeated but brought up to date and augmented with descriptions of pactical set-ups and manipulations.

FILTERS

Allen filters.† These filters are similar to the Mandler filters except that they are made of unglazed porcelain instead of diatomaceous earth. They possess a finer porosity than is possible in filters made of diatomaceous earth and thus have a higher retaining power. They are used mainly for the filtration of water.

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* Read before the Technicians' Institute, Temple University School of Medicine, Philadelphia, Pa., April 11–13, 1938. Received for publication May 6, 1938.

† Manufactured by The Allen Filter Co., Toledo, Ohio.

Berkefeld filters. The filters which enjoyed the greatest usage in bacteriological laboratories until the World War were the various types and sizes of Berkefeld filters. These filters are manufactured from Kieselguhr, the siliceous remains of microscopic plants, known as diatoms (diatomaceous earth). The filters are available in sizes ranging from 3 cm. in length by 1.5 cm. in diameter to 25 cm. by 5 cm. They are graded according to pore size by testing the rate at which filtered water passes through them under a given pressure. The Berkefeld N (normal) filter is the grade most commonly used for bacteriological work. The mean pore diameter is about 5 to 7μ. Berkefeld W (dense)

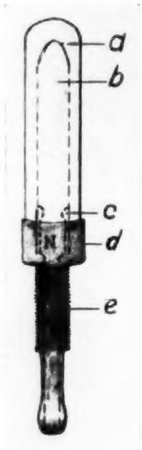

FIG. 1. BERKEFELD FILTER, ½ ACTUAL SIZE

a, inverted cylinder, closed at one end, of diatomaceous earth. *b*, bore of filtering cylinder. *c*, cement joining cylinder to threaded stem *e* and also to metal collar, *d*. The collar bears the letters "V", or "N" "W" to designate the range of pore size. In this particular size of Berkefeld filter, 3.5 to 4 cc. of liquid are retained by the filter, itself.

filters are sometimes used in bacteriological work but the rate of filtration is slow because the diameter of the pores is smaller than in the case of the N, the mean being about 3 to 4μ. The Berkefeld V filter is very seldom used to withhold bacteria; its chief use being to remove coarse particles from suspension. It is often used to clarify material before filtration through N or W filters. The mean pore diameter of the V filter is about 8 to 12μ. Figure 1 shows a Berkefeld filter of convenient size for the filtrations usually required of the bacteriological laboratory.

Chamberland (Chamberland-Pasteur) filters. This type of filter was the first to be introduced into bacteriological work. The shape, more than any other

feature, is responsible for their not enjoying greater usage. One very desirable feature, however, is their range in porosities. The filters are labeled as follows: L1, L1 bis, L2, L3, L5, L7, L9, L11, and L13, in order of increasing fineness. Filters of L3 porosity and finer will withhold bacteria. There are also filters labeled "F" and "B" for water filtration. Their porosities correspond to L5 and L7, respectively. Chamberland filters are also made with a collar at the

Fig. 2. Chamberland Filter, ½ Actual Size

The lower, shaded portion is of unglazed porcelain and is that portion of the filter which is concerned with filtration. The upper, shiny portion is of glazed porcelain. Near the top of the glazed portion is the label, designating the porosity.

open end and the tip shaped so as to be conveniently attached to rubber tubing but the most common form is that shown in figure 2.

Jenkins filter. In 1927 Jenkins[13] devised a filter for filtering small quantities of material. The filter is known to withhold 24-hour broth cultures of *Bact. coli*, *Bact. prodigiosum*, *Bact. typhosum*, *Staphylococcus aureus* and an enterococcus. Jenkins claimed that only 0.4 cc. of material was retained by the filter when using a procelain block 9 mm. in diameter and 110 mm. in length.

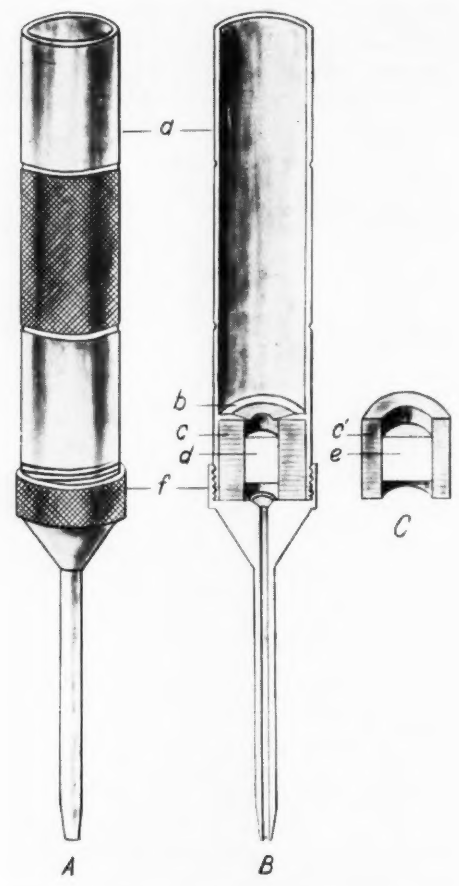

Fig. 3. Jenkins Filter, ½ Actual Size

A, external view of filter. *B*, cross section view. *C*, soft rubber sleeve and larger porcelain block. *a*, mantle, capacity about 55 cc. *b*, metal diaphragm. *c*, *c'*, soft rubber sleeves. *d*, smaller filtering block of porcelain, 10 mm. in diameter and 12 mm. high. *e*, larger filtering block of porcelain, 15 mm. in diameter and 12 mm. high. *f*, metal portion carrying delivery tube. When this portion is screwed onto the mantle, pressure is exerted against the rubber sleeve which then presses tightly against the walls of the mantle and also against the porcelain filter block.

Using the American made Jenkins filter* and the 10 mm. porcelain block, we have found that slightly more than 1 cc. of material is lost during the filtration process. A strong suction is required for filtration and even then filtration is slow. We have found that about one hour is required to filter 50 cc. of distilled water through either the 10 mm. or 15 mm. porcelain blocks under a suction of 700–745 mm. of mercury. Figure 3 illustrates the Jenkins filter in detail.

Mandler filters.† These filters are very similar to the Berkefeld filters in appearance and function. They are manufactured from kieselguhr, asbestos and plaster of Paris. They were developed in America to replace the Berkefeld filters when the supply of the latter was stopped during the World War. Mandler filters are available in three grades, representing differences in pore size. Filters of "preliminary" grade allow air to pass through at pressures of 2 to 5 lbs. per sq. in., corresponding to the Berkefeld V filters, and are not intended to withhold bacteria. Their main use is to remove coarse particles from suspension. Filters of the "regular" grade allow air to pass through at pressures of 6 to 9 lbs. and the "fine" grade, 10 to 16 lbs. They correspond to the Berkefeld N and W filters, respectively.

The care, operation and testing of Mandler filters is essentially the same as that for the Berkefeld filters. In addition to being similar in all other respects their being more durable, cheaper in cost and more readily obtainable justifies their replacement of the Berkefeld filters.

Plaster of Paris filters. Of more theoretical than practical importance are the plaster of Paris filters which were devised by Kramer. These filters carry an electric charge (+) opposite to that carried by other types of bacterial filters. If needed, the filters can be made easily in the laboratory by following the directions quoted by Mudd.[23]

Seitz filter. A type of filter which employs a disc of asbestos for the filtering mechanism and which is enjoying wide usage in bacteriological and chemical laboratories is the Seitz filter, Uhlenhuth model,[28] as shown in figure 4. The reliability of this type of filter was demonstrated by earlier workers and later by Larkum.[17] The Seitz filter presents many desirable features; being made of metal, it is not subject to breakage by careless handling or by the usual laboratory accidents. It requires very little time to prepare it for use—simply insert a new asbestos disc. The Uhlenhuth model, constructed so as to use either suction or pressure, is a distinct advantage. The larger size, one which holds about 100 cc. of material, is the most popular although a smaller size, holding about 25 cc., is available. The asbestos disc for the larger size Seitz filter retains about 5 cc. of material. The discs, Seitz, EK (Entkeimende), although renewed after each filtration, are inexpensive, and, taking into con-

* Manufactured by The Fisher Scientific Co., 711–723 Forbes St., Pittsburgh, Pennsylvania.

† Manufactured by The Allen Filter Company, Toledo, Ohio.

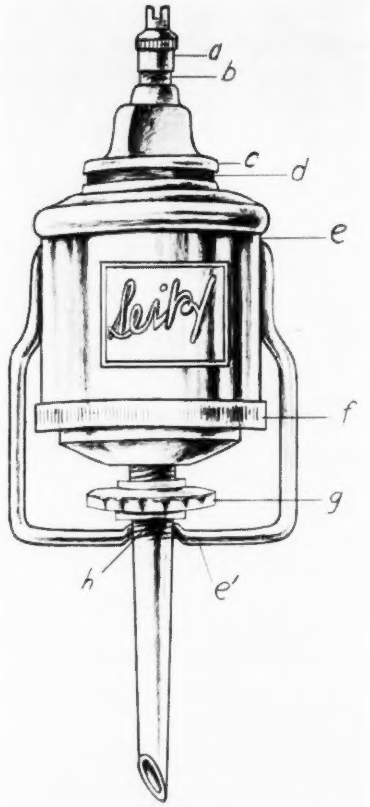

FIG. 4. SEITZ FILTER, UHLENHUTH MODEL, ½ ACTUAL SIZE

a, cap. *b*, stem containing a valve. *a* and *b* are similar to the valve and cap on a bicycle tire. *c*, top, which screws into top portion of mantle, *e*. The rubber washer, *d*, insures an air-tight connection between *c* and *e*. *c* and *d* are only used when it is desired to apply pressure above the material in the mantle. The mantle, *e*, has a capacity of about 100 cc. *f* is the base which carries the burr, *g*, on the threaded stem, *h*. To assemble the filter, a small wire screen is placed on the flat, interior surface of *f*; on the screen is placed a fresh Seitz EK asbestos disc, hard surface against the screen. The two parts, *e* and *f* are then put together as shown, with the asbestos disc being firmly pressed against the rims of the two parts. The burr, *g*, is then turned until it presses firmly against *e'*.

sideration the long period of usefulness of the metal parts, the operation of the filter is very economical. Faults of the earlier models of Seitz filters were quite often overcome by such little changes as those proposed by Larkum.[18]

In case it is necessary to prevent material from coming in contact with metal, a Seitz filter can be constructed of Pyrex glass, as proposed by Bruce.[5] Very small filters for special work can often be constructed like that described by Johnston.[14]

Sintered (fritted) glass filters.[20] Filters made solely of resistant glass are the nearest approach to the ideal filter. This type of filter is manufactured from Jena 20 glass, ground to particles of uniform size and the powdered glass heated in a mold to the sintering point without the addition of any other substance. The discs are then fused into glass vessels of suitable shape and capacity. The filters* are then tested for filtering speed and pore size (average pore diameter of no. 5 porosity sintered glass disc is about 0.5 to 0.75μ). The filters can be chemically cleaned as well as sterilized. After chemically cleaning with sulfuric acid containing a little sodium nitrate and sodium chlorate, all traces of acid must be washed out of the filter with distilled water. Hofmann[11] stated that the titers of some sera were reduced by as much as 65 per cent as the result of filtration. It has been our experience that *agglutinin* and *precipitin* titers are unchanged as a result of filtration through clean, sterile glass filters. Figure 5 illustrates a sintered glass filter of convenient size for bacteriological work.

Dialyzers. The process of removing crystalloids from colloids by allowing the former to pass through a semi-permeable membrane into pure water, while the colloids are left behind, is known as *dialysis.* This process of separation is quite often necessary in biological work. Parchment or cellophane make satisfactory membranes. The material to be dialyzed may be placed in a bag, made by gathering together the edges of a sheet of the dialyzing membrane, and suspended in running water or the membrane may be firmly tied across an opening in a suitable glass vessel. A more practical way is to use tubular cellophane.† This material is available in various grades and widths, so a satisfactory size may be chosen for the volume of material to be dialyzed. The cellophane is moistened with distilled water and a knot tied at one end of the tube. The material to be dialyzed is then placed in the closed tube of cellophane and a knot tied at the top of the tube, close to the level of the liquid. The filled tube is then submerged in running distilled water and dialysis carried on until the material is free of electrolytes.

Electro-dialyzers. In order to increase the dialyzing effect and thus shorten the time required for dialysis, an electric current may be applied so as to obtain

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* United States Agents, Fish-Schurman Corporation, 250 East 43rd Street, New York, N. Y.

† Obtainable from the Visking Corporation, 6733 West 65th Avenue, Chicago, Ill.

a combination of dialysis and electric transport, a process known as *electro-dialysis*. An apparatus for electro-dialysis can very readily be made according to the directions by Czarnetzky.[8]

Ultra-filters. The process of dialysis is relatively slow. If the intramicellar liquid in the membrane is forced through by means of pressure, the process of

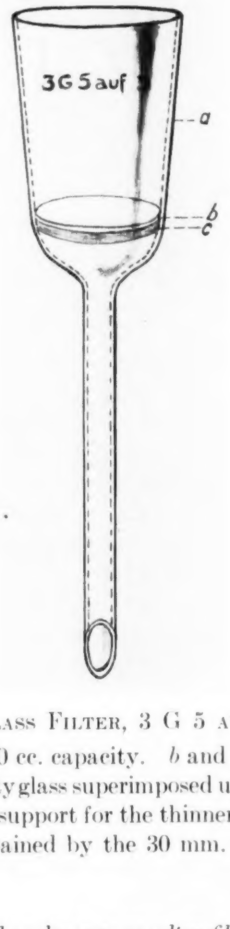

FIG. 5. SINTERED GLASS FILTER, 3 G 5 AUF 3, ½ ACTUAL SIZE

a, glass funnel of about 30 cc. capacity. *b* and *c* are the sintered glass disc; *b* being a layer of no. 5 porosity glass superimposed upon *c*, a layer of no. 3 porosity glass, the latter serving as a support for the thinner layer of finer porosity glass. The amount of material retained by the 30 mm. sintered glass disc is about 0.45 cc.

dialysis is hastened and is then known as *ultra-filtration*. Unglazed porcelain vessels of special design or alundum thimbles are used as a supporting mechanism for the collodion membrane which is usually made from collodion dissolved in glacial acetic acid or in alcohol-ether mixture. For directions in preparing satisfactory collodion membranes and setting up an ultra-filtration apparatus see Bronfenbrenner,[3, 4] McBain and Kistler[19] and Asheshov.[1]

Electro-ultrafilters. The slow process of ultrafiltration may be hastened by the passage of an electric current through the material being ultrafiltered. The process of dialysis is thus aided by pressure and by electric transport. The electric current not only aids in the transport of the electrolytes but actually prevents the collodion membrane from clogging. Apparatus for electro-ultrafiltration has been described by various authors[3] and can readily be constructed[7] in the laboratory.

MANTLES

Some filters (Jenkins, Seitz, sintered glass) are so constructed as to provide a storage space or reservoir above the filtering mechanism for the material to

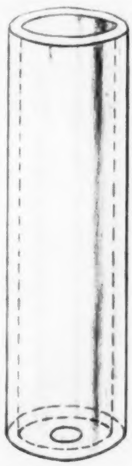

FIG. 6. GLASS MANTLE, USUALLY OF PYREX GLASS, WITH A HOLE IN THE BOTTOM FOR THE STEM OF THE FILTER

be filtered. Other filters, such as the Allen, Berkefeld, Chamberland and Mandler filter candles, require assembling with a mantle in order to hold the material around the filter. The most common type of mantle is that shown in figure 6. The glass mantles are available in a variety of sizes, depending on the size of the filter which is to be placed inside them and the amount of material which it is desired for them to hold.

When filtering small amounts of material or to keep the filter completely covered until practically all of the material is filtered, the glass mantles can be made smaller in diameter, as suggested by Schereschewsky[27] and Rose[26].

Another plan for keeping the filters completely covered until practically all of the material has passed through, is to cover the filter with a glass thimble as shown in figure 7.

A very convenient method, and one which is just as efficient as a glass thimble or narrow mantle for keeping the filter covered with material is to have the material to be filtered contained in a test tube (about 25 x 200 mm. tube, without lip); remove the cotton plug from the tube, place the sterile filter, assembled with appropriate mantle, inverted into the tube, invert the tube and filter assembly and the test tube then serves as a thimble and practically all the material to be filtered is held in contact with the filter until it has passed through.

RECEPTACLES FOR THE FILTRATES

The greatest pit-fall in the technic of bacterial filtration is the collection and handling of the filtrate so as to maintain sterility.

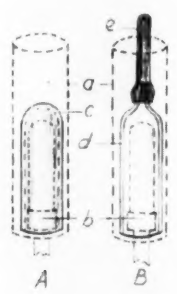

FIG. 7. GLASS THIMBLES FOR FILTER CANDLES

A. *a* is the glass mantle. *b*, the filter candle. *c*, the inverted glass thimble. In *B*, *d* is the same type of thimble except that the end has been drawn out to a tip and a piece of rubber tubing, *e*, attached. After the material to be filtered has been poured into the mantle, suction is applied to *e* with a pipette until the thimble *d* is filled with liquid. *e* is then closed with a clamp. *B* is the more satisfactory of the two arrangements.

The size, shape and nature of the receptacle for the filtrate depends upon the volume and the disposition which is to be made with the filtrate.

Test tube with side arm. For receiving filtrates of small volume, the most convenient receptacle is a test tube with a side arm. These tubes can be purchased from almost any scientific supply house or easily made by joining a piece of glass tubing onto the side of a test tube. One has a choice of as many different sizes as there are different sizes of test tubes. The size which we have found most convenient for general laboratory work has been the 30 mm. by 200 mm. tube, as shown in figure 8. It very conveniently holds filtrates up to vol-

umes of 80 cc. This type of receptacle can be used with Berkefeld, Jenkins, Mandler, Seitz or sintered glass filters; the filters being tightly fitted into the

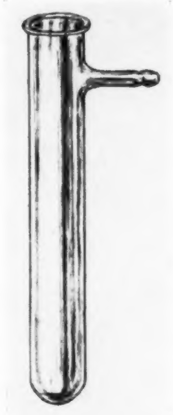

FIG. 8. TEST TUBE WITH SIDE ARM

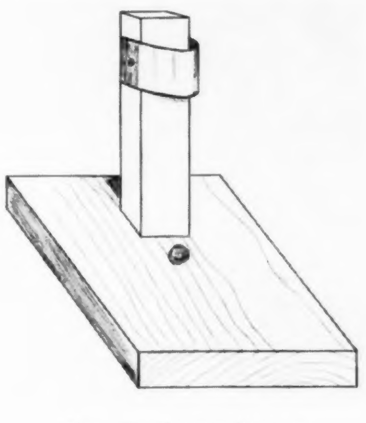

FIG. 9. FILTER STAND

To a wooden base is fastened a block of wood of suitable size. Near the top of the block of wood is placed a strip of soft metal, such as copper, in order to hold the tube in a vertical position. A depression is made in the base in order to prevent the base of the tube from shifting.

top of the tube by means of a one-hole rubber stopper and the side arm plugged with cotton.

For support during filtration, the complete filter assembly can be clamped to a ring stand for support or rested in some sort of a home made holder, as

FIG. 10. MUDD FILTRATE MEASURING CYLINDER AND DELIVERY TUBE WITH PROTECTIVE APRON

a is the graduated cylinder with side arm, *d*, for connection to suction pump. Side arm should be plugged with cotton at *e*. *b* is the delivery tube with protective apron, *c*, which also must be plugged with cotton. The rubber tubing which joins the delivery tube to the cylinder must be closed at *f* with a pinch-clamp during the filtration process.

pictured in figure 9. We have found the latter very convenient, as only one hand is required to remove the filter assembly from its support or to replace it.

*Filtrate measuring cylinder.** Mudd,[22] in 1927, described a filtration apparatus which makes use of a graduated cylinder for receiving the filtrate. This receptable, figure 10, is very convenient when it is desired to measure the volume of the filtrate, draw off measured quantities of the filtrate or to dispense the filtrate aseptically into sterile containers.

Technic of Gee[9]. For filtering fairly large volumes of material into flasks, and when it is intended to leave the material in the flasks, the technic proposed

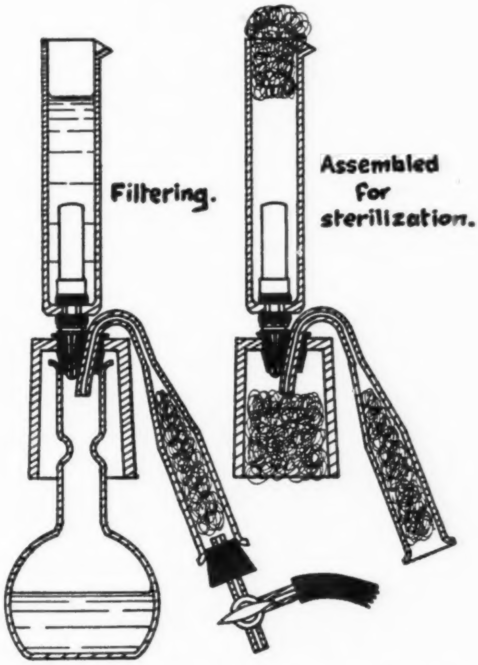

FIG. 11. FILTER ASSEMBLY AS PER THE TECHNIC OF GEE (FROM THE ORIGINAL DESCRIPTION)

At the right, the apparatus is ready for sterilization; at the left the apparatus is assembled to a Florence flask, the neck of which has been somewhat constricted as a preliminary step to subsequent sealing.

by Gee can be used. Figure 11 is a reproduction of the diagram from the original description.

Technic of Grubb[10]. When it is necessary to filter fairly large volumes of materials and later transfer the filtered material aseptically to other containers,

* Obtainable from The Arthur H. Thomas Co., Philadelphia, Pa.

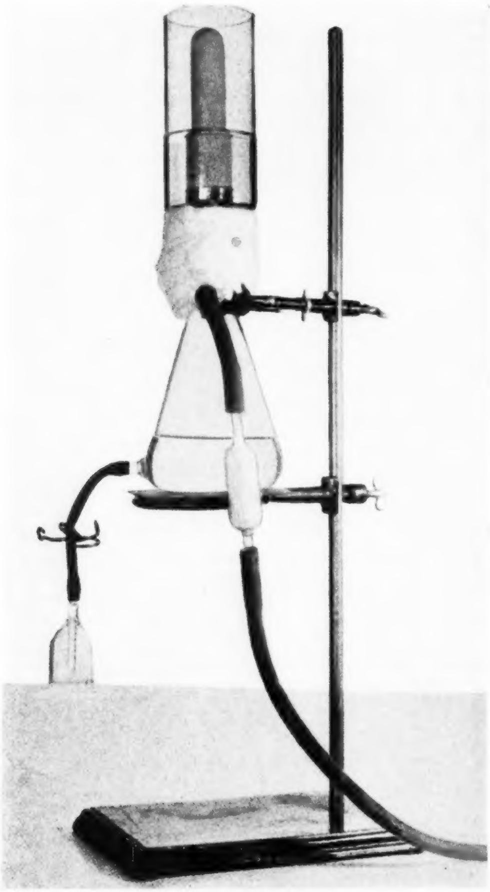

FIG. 12. FILTER ASSEMBLY AS PER THE TECHNIC OF GRUBB (FROM THE ORIGINAL DESCRIPTION)

The filtration flask with a side arm near the top for applying suction and a similar side arm near the bottom for drawing off the filtrate can be purchased from the Corning Glass Works, Corning, N. Y., or made by a glass blower from an ordinary Pyrex filtration flask. The delivery tube with protective apron, as pictured in figure 10 for the Mudd filtration apparatus, is perfectly satisfactory for use in this set-up.

as for example, tubing of culture media, the technic described by Grubb can be used. Figure 12 is a reproduction of the assembly as originally pictured.

Test tube with vent. For collecting filtrates of very small amounts, a test

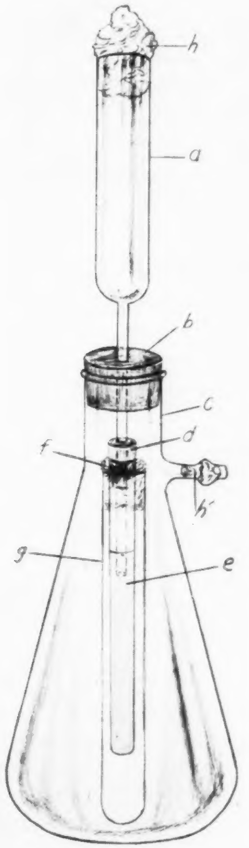

FIG. 13. CHAMBERLAND FILTER ASSEMBLED, $\frac{1}{4}$ ACTUAL SIZE

Tube *a* with a narrow tube joined at the bottom, serves as the mantle for holding the material to be filtered. The narrow tube is tightly fitted to the Chamberland candle, *e*, with a one-hole rubber stopper, *d*. A thin strip of cotton, *f*, is wrapped around the top of the candle so that the candle fits snugly into the large test tube, *g*, which receives the filtrate. The assembled candle is then placed in the filtration flask, *c*, which can be closed tightly by the rubber stopper, *b*. The two openings, *h* and *h'*, are plugged with cotton and the assembly sterilized. When cool, it is ready for use. A layer of cotton can be placed in the bottom of the filtration flask, on which the test tube can rest during filtration. Any vessel with a large enough opening and suitable for withstanding suction, may be used in place of the filtration flask.

tube with vent has been proposed. This can be used only when the filters have a long stem and practically the only ones so constructed are the Jenkins and sintered glass filters. It is more cumbersome than the tube with side-arm and possesses no advantages for which it can be recommended.

The choice of a satisfactory mantle or suitable receptacle for the filtrate in connection with Chamberland filters is not as easy as with the other types of filters. An arrangement similar to that described by Kolmer[15] may be used or the set-up illustrated in figure 13 is very satisfactory.

CARE AND HANDLING OF FILTERS

New filters should be numbered or in some way marked for identification. When more than one filter is in use (which is almost always the case), it is often important to know which filter was used for filtering a particular substance and how the filter functioned during previous and subsequent filtrations.

Filters should not be allowed to come in contact with oily substances or surfaces as Holman and Krock[12] found that oily substances rendered filters more permeable to bacteria.

Porcelain and kieselguhr filters, which are used a great deal, should be heated occasionally to redness in a muffled furnace to eliminate the organic material which was not removed by washing with water.

New filters should be immersed in distilled water, except for the open end, and allowed to soak for at least a few hours. Brush under a gentle stream of water with a soft brush, such as an old tooth brush. Berkefeld filters are very brittle and will not stand rough handling. Mandler and Chamberland filters may be brushed with a stiff brush. The brushing is to remove the powdery material which is present on new candles, so that it will not pass through into the first filtrates. Filter several hundred cubic centimeters of distilled water through the filter, then reverse the filter and force water through the filter from inside outward.

Test the wet filters by air pressure by attaching the stem to rubber tubing connected to the compressed air line and completely submerge the filters in a glass vessel containing distilled water. Gradually turn on the air and note the pressure at which air passes through the filter. The appearance of a coarse stream of bubbles at a relatively low air pressure indicates an imperfection in the filter. This test in addition to detecting imperfections also gives some indication as to the porosity of the filter. If the air test is satisfactory, the filter may be assembled and sterilized.

Used filters, if only soiled with a coagulable material such as serum and not contaminated with infectious cultures, should be washed in both directions with distilled water, to remove the coagulable material from the pores, boiled for 15 minutes in clean distilled water, washed in both directions with distilled water, tested with air pressure and then reassembled for use.

Used filters, if contaminated with infectious material, should be placed in

clean distilled water, brought to a boil and boiled for 15 minutes. The candles may then be safely handled. They should be washed with distilled water from the inside outward then thoroughly washed by filtering a few hundred cc. of distilled water through them in the usual direction.

In the case of the Seitz filter, if contaminated with infectious material, the whole filter may be placed in a large vessel of water and sterilized by boiling. The filter may then be taken apart, the used asbestos disc discarded, the metal parts washed with distilled water, a new asbestos disc inserted, the metal parts reassembled and the filter is ready for sterilization and use.

The pores in the sintered glass filters are fine and readily become clogged with material. Washing with water is not sufficient cleaning. Usually it is desired to have the filters chemically clean so they are cleaned by placing in concentrated sulfuric acid containing a small amount (2 or 3 grams) of sodium nitrate and sodium chlorate, heated to 90°C. and allowed to stand overnight. The filters are then thoroughly rinsed with water and a few hundred cc. of distilled water filtered through them. It is very important to remove all traces of the acid. To make sure that the acid is completely removed, the filtered distilled water may be tested from time to time with a pH indicator. The rate of filtration of the distilled water will often indicate any imperfections or the filters may be tested by air pressure.

ASSEMBLING OF BACTERIAL FILTERS

The Berkefeld and Mandler types of filters have to be assembled with a mantle, as shown in figure 6. Onto the stem of the filter, close to the collar, is placed a rubber washer. The stem of the filter is then inserted through the hole in the bottom of a glass mantle of suitable size and a second rubber washer placed on the stem and shoved tight against the bottom of the mantle. A metal washer is then placed against the rubber washer and the fittings tightened by means of a burr. A one-hole rubber stopper is then placed on the stem and fitted into the mouth of the filtrate receptacle, such as a test tube with a side arm, flask, etc. When large filters and mantles are fitted to tubes, such as the side arm tube or Mudd filtration apparatus, the set-up is apt to be top heavy and should be secured in position by tying with string. The filter may be covered with one of the glass thimbles as shown in figure 7 and the top of the mantle plugged with cotton as well as the side arm in the filtrate receptacle.

After repeated autoclaving the rubber stoppers assume the shape of the opening in the filtrate receptacle and may not retain a tight fitting. To guard against contamination of the filtrate by the leakage of air into the filtrate receptacle around the rubber stopper, the connection should be wrapped with cotton, as shown in figure 12.

Jenkins, Seitz and sintered glass filters need only to be connected to a suitable receptacle by means of a rubber stopper.

After the filters are completely assembled, and all openings stoppered with

cotton, the assembly should be wrapped in paper (a few thicknesses of newspaper or wrapping paper). The type, size, grade and number of the filter should be marked on the outside of the wrapper.

STERILIZATION

The wrapped, moist filters should be sterilized in the autoclave at 120°C. for 30 minutes. The wrapping also prevents the filters from cooling too rapidly when removed from the autoclave.

FILTRATION

The material to be filtered should be freed as much as possible from gross particles so that the pores of the bacterial filter will not become clogged and filtration slowed or even stopped. Centrifugation, filtration through filter paper pulp, asbestos pulp, filter paper, Berkefeld V, Mandler preliminary or coarse sintered glass filters may be employed to clarify, partially, the liquid.

After the filter has become thoroughly moistened with the material to be filtered, suction or pressure is applied. One should not use excessive suction or pressure for filtration—only enough to obtain a reasonable rate of filtration.

No matter how efficient a bacterial filter may be in withholding bacteria, if left in contact with the filter long enough the bacteria will pass through (Perinei[25], Hofmann[11] and others). Cronkright and Miller[6] have recently shown that bacterial filters in water lines have brought about an increase in the bacterial count by as much as 34,900 per cent. The time required for their penetration is dependent upon the porosity, permeability and size of the pores of the filter, size and morphology of the microorganisms, the chemical nature of the filtering fluid and physical factors, such as temperature. One should not, therefore, filter too much material through one filter at a given time or continue the process too long. If the volume to be filtered is large or the rate of filtration slow, it is better to use several filters instead of one.

The filter assembly should be connected to the suction line by means of a T-tube, the side arm of which is closed with a short piece of rubber tubing and a pinch-clamp. Several filter assemblies may thus be connected to the same suction line at the same time and any one of them disconnected without disturbing the vacuum being applied to the other filters. To disconnect a filter from the suction line, clamp the rubber tubing between the T-tube and source of vacuum. Air is then let into the filter assembly very gently by slowly releasing the clamp on the side arm of the T-tube. By allowing the air to enter slowly, the cotton plug in the side arm of the filtrate receptacle is prevented from being drawn into the vessel and thus contaminating the filtrate.

The amount of suction or pressure employed for each filtration should be noted. A 3-mouth Woulff bottle should be inserted in the vacuum line for a trap. Usually the suction is created by a filter pump attached to the water line. The Woulff bottle should, then, be inserted in the line near the filter

pump, the connection from the filter pump to the Woulff bottle extending nearly to the bottom of the bottle. In case any liquid collects in the Woulff bottle it will then be drawn out through the filter pump and not left to accumulate in the trap. The connection from the vacuum line, to which the various filters are attached, to the Woulff bottle should extend just through the top of the bottle. To the third opening in the Woulff bottle is connected a manometer to record the amount of vacuum.

CONTROLS ON FILTRATION

Suitable amounts of the filtrate should be seeded into appropriate liquid and onto solid culture media and incubated several days to determine the sterility of the filtrate. A sample of the filtrate should also be incubated.

In the past it has been customary to add a few cubic centimeters of a young broth culture of *B. prodigiosus* (*Chromobacterium prodigiosum*) to the material to be filtered. If none of the organisms grew out in the filtrate, the filtrate was considered sterile. During the last decade we have learned a good many things about the variation in morphology of bacteria. We now know that bacteria are capable of existing in many different morphological forms in addition to the "normal" vegetative form which we are so accustomed to seeing. Even the control organism *Chromobacterium prodigiosum* is known to undergo variations, the same as other microorganisms, so the introduction of a group of unknown factors into an already complicated system for the purpose of a control is open to criticism.

DISCUSSION

Bacterial filtration depends upon several factors. Not as important a factor as one might expect is the sieve-like action of the filter itself, as Beckhold[2] has shown that the diameter of the pores of the filter must be 8 to 15 times the length of the microorganism, if a single microorganism is to pass through. The size, shape and number of pores per unit area has also been ascertained by Peragallo[24]. More important than the size of the pores is the tortuosity of the channels through the filter. The chemical character of the liquid passing through the filter has a marked influence on the permeability of the filter, as for example, liquids containing large amounts of nutritive substances may be partially adsorbed onto the walls of the filter and thus bring about a decrease in the pore diameters and of the intergranular spaces. The electric charge[21,16] must also be taken into account when selecting the proper filter because undoubtedly

it causes certain materials, even the bacteria themselves, to be adsorbed onto the walls of the filter.

SUMMARY

The various types of bacterial filters now available are listed, as well as the method for assembling each type of filter.

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ADDENDUM

Since this material went to press a new style of glass mantle for Berkefeld and Mandler filters has been described by C. A. Hoag in *Chemist Analyst*, **27**, No. 2, 40 (1938). A new style of Seitz filter has also been made available by the Becton, Dickinson & Co., Rutherford, New Jersey. This miniature Seitz filter, called B-D Swinny Filter Adapter by the manufacturers, can be employed with Luer-Lok syringes and is adaptable to the filtration of small amounts of fluid.

TECHNICAL PROCEDURES IN BLOOD CHEMISTRY FOR THE SMALL HOSPITAL LABORATORY*

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Even trained biochemists sometimes meet problems in the handling of routine analyses that tax their ingenuity; so it is no reflection upon medical technicians that they occasionally encounter difficulties. It is hoped that the following suggestions upon general and specific procedures may assist technicians, especially those in small laboratories. No claims for originality are made, though all suggestions are based on experience.

Cleaning glassware. A 1 per cent solution of sodium metasilicate (obtainable from the Philadelphia Quartz Co. under the name of "Metso") is useful for soaking pipets and other glassware that has contained protein solutions. They are then rinsed with tap and with distilled water, and dried by running a few drops of acetone into the tips and allowing them to stand vertically, tip up, on a paper towel. In general, acetone can replace the old-established alcohol followed by ether in drying glassware. Once a week the pipets should be soaked in a chromic acid solution to remove alkali-insoluble material. It is a mistake to depend entirely upon chemical reactions to clean glassware. In many cases there is nothing better than soap and water or one of the well-known kitchen utensil cleansers applied vigorously with a brush.

Occasionally, even after vigorous cleaning, a buret, especially an old one, refuses to drain cleanly. This annoying condition can be corrected as follows: Suspend technical sodium fluoride in an equal weight of water, and acidify with an equal volume of concentrated hydrochloric acid. A precipitate of sodium chloride will occur. Pour the mixture into the buret and allow it to remain for a few minutes. Then remove, rinse, and scrub with a long-handled brush. If drainage is not perfect, return the fluoride solution to the buret for a few more minutes. Surface layers of old glass are removed, leaving a new surface. Hence treatment should not be too long-continued, or appreciable changes in capacity may occur. Since there is free hydrofluoric acid present,[1] great care should be taken to avoid contact of the liquid with the skin.

Protein-free blood filtrates. Technicians seldom have trouble with these, provided they follow directions accurately. Sometimes only 1 or 2 cc. of blood

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are obtainable. If this small amount of blood is placed in an oxalate tube containing enough oxalate for 10 or 15 cc., the excess oxalate reacts with the sulfuric acid when a Folin-Wu filtrate is prepared, to form oxalic acid, and the result is as if too little acid were present. The filtrate comes through the paper colored. Of course the proper procedure is to adapt the amount of oxalate to the amount of blood (about 2 mg. per cc.), but it may be just in the most important cases that no more blood is available. Then the filtrate can be saved by adding small drops of approximately N or $\frac{2}{3}$ N sulfuric acid and refiltering through the same paper until the filtrate is colorless.

The Haden modification for making Folin-Wu protein-free filtrates[2] is a useful one. One volume of blood is laked with 8 volumes of N/12 sulfuric acid, one volume of 10 per cent sodium tungstate is added, and after vigorous shaking the mixture is filtered.

Micro-Kjeldahl digestions. In carrying out total protein determinations, where only sulfuric acid plus a trace of copper sulfate is employed as the digesting agent, incomplete oxidation of the organic material will give high results for nitrogen upon Nesslerization. Time can be saved and complete oxidation secured if a stronger oxidizing agent is used. Heating is carried on until the preliminary dark color has begun to fade to a brown. Then the tube is allowed to cool somewhat, and a drop of 30 per cent hydrogen peroxide is added.[3] Heating is resumed and is carried on until the solution is entirely clear (at least two or three minutes). If the hydrogen peroxide contains nitrogenous materials, a correction should be made (run blank).

When a phosphoric acid-sulfuric acid digestion mixture is used, as in the determination of non-protein nitrogen, one sometimes encounters a white precipitate, caused by the action of the phosphoric acid on the glass. This can usually be avoided by using a very small flame after white fumes begin to appear, the heating being then continued as short a time as possible, preferably not more than two minutes.

Nesslerization. This important procedure is employed under especially adverse circumstances in clinical laboratories, where ammonia is frequently not distilled off, but is determined directly in the enzyme mixture or digestion mixture in which it is formed. Alkaline Nessler reagent should be made up and kept in two bottles, each of which contains enough supply for a week or ten days. As soon as one bottle is emptied, it should be filled with a fresh supply, made up from the stock solution, and set aside, well stoppered, for a week or so, while the supply in the other bottle is being used. Standing allows mercurous compounds, and possible contaminating nitrogen compounds to settle to the bottom. The clear supernatant fluid is removed as needed by decantation.

It is by now well known that a few drops of 1 per cent gum ghatti solution added to the unknown, and of course also to the standard, before Nesslerization, usually prevent clouding or precipitation in the solutions.[4] The gum acts as a protective colloid to prevent aggregation or clumping of submicroscopic par-

ticles that produce the color. A good rule is to add 1 drop of gum ghatti solution for each 5 cc. of solution in the Nesslerized mixture.

Finger tip blood. Sometimes it is difficult or impossible to obtain blood from the patient's veins. In such cases, the doctor may be willing to have blood sugar determinations performed upon finger tip blood by a micro method. Finger tip blood is capillary or arterial blood. In the fasting state in normal individuals, and both in the fasting and in the post-absorptive state in diabetic individuals, finger tip blood has about the same glucose content as does venous blood. On the other hand, when a normal individual is absorbing glucose, his arterial blood may contain 30 to 70 mg. per 100 cc. more sugar than does his venous blood. Therefore the doctor should certainly be notified if it is necessary to use finger tip blood in a glucose tolerance test, in order for him to be able to interpret correctly the slightly different type of curve that is obtained.

A bayonet-type removable knife blade (Baird-Parker No. 11) is a particularly efficient instrument for making finger punctures, and is far superior to the usual needle or automatic lancet. Its sharp edge cuts the skin cleanly without tearing, leaving a puncture which bleeds freely and heals with a minimum of soreness. The finger should be squeezed and released alternately, in order to allow blood to flow to the tip. Steady pressure is less effective. For good flow in a baby, the punctured part, usually the heel, should be kept below the level of the rest of the body.

One of the most convenient and reliable, and least troublesome, micro methods for blood sugar is that of R. B. Gibson,[5] which is not mentioned in most textbooks. Several of the reagents used in his method are the same as those for the Folin-Wu method. The reagents do not decompose rapidly, and the color obtained is easy to match, which is more than can be said for Folin's ferricyanide micro method. Two cc. of concentrated sulfuric acid are pipetted into 100 cc. of water. Five cubic centimeters of this diluted acid are added to 43 cc. of 1.25 per cent sodium tungstate. The mixture must be made freshly on the day used. Into a centrifuge tube are placed 4.8 cc. of the tungstic acid mixture. Finger tip blood is drawn into a dry pipet calibrated to contain 0.200 cc., and the blood is blown into the tungstic acid solution in the centrifuge tube, the solution being sucked up several times to rinse the pipet. The tube is stoppered, shaken, allowed to stand 15 minutes, and centrifuged. Two cubic centimeters of the clear fluid are transferred to a Folin-Wu sugar tube which has been calibrated at 10 cc. Two cubic centimeters of Folin-Wu copper solution are added, and the tubes are immersed in boiling water for 6 minutes and then cooled. Two cubic centimeters of Benedict's arsenic-phosphotungstic acid reagent containing 5 per cent formalin* are added, and the

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* Dissolve 100 grams sodium tungstate in 600 cc. of water. Add 50 grams of arsenic pentoxide, 25 cc. of 85 per cent phosphoric acid, and 20 cc. of concentrated hydrochloric acid. Boil 20 minutes, cool, and dilute to 1 liter. Remove 100 cc. at a time to a small bottle and add 5 cc. of 40 per cent formaldehyde.

tube contents are well mixed by shaking, and diluted to 10 cc. The standard, consisting of 2 cc. of 0.01 per cent glucose (the regular Folin-Wu dilute standard), is treated similarly, but is diluted to 25 cc. The reaction does not give results which conform to Beer's law; so Gibson and an associate worked out a table of empirical results that apply when the standard is set at 10 mm. and the unknown is read against it. For readings of the unknown not greater than 18 mm., the empirical table gives results corresponding fairly well to the formula

$$G = \frac{1000}{U} - 3\,(U - 7)$$

where G is milligrams of glucose per 100 cc. of blood, and U is the reading of the unknown in millimeters when the standard is set at 10 mm. This method gives lower values for blood sugar than does the Folin-Wu method. According to Gibson the filtrates contain less non-glucose reducing material.

Urea clearance. Simplification of procedure that does not sacrifice accuracy is always welcome. Van Slyke and his associates state[6] that it is desirable to omit separate determination of ammonia in urine when determining urea clearance. The ammonia plus urea can be determined in one step and calculated as urea, since blood urea is the source of urinary ammonia.

Cerebrospinal fluid protein. Pandy's test in our hands never fails to show turbidity when the total protein content of spinal fluid is as great as 50 mg. per 100 cc. For accurate quantitative determination of the protein content, the following method[7] is useful: In a conical centrifuge tube place 1 to 3 cc. of spinal fluid, depending inversely on the intensity of the Pandy test. Make to 5 cc. with water. Add 1 cc. of 10 per cent sodium tungstate, mix, add 1 cc. of $\frac{2}{3}$ N sulfuric acid, and mix again. Centrifuge and decant supernatant fluid. Dissolve the precipitate in 0.5 cc. of 10 per cent sodium tungstate and dilute to 5 cc. with water. Add 0.5 cc. of $\frac{2}{3}$ N sulfuric acid, mix, and centrifuge. Decant supernatant fluid. Dissolve precipitate in several drops of approximately 0.1 N NaOH, and transfer quantitatively to a micro-Kjeldahl digestion tube with a medicine dropper drawn out to a capillary, rinsing centrifuge tube and capillary several times with two or three drops of water. Digest the protein solution in the usual manner, using the non-protein nitrogen digestion mixture (sulfuric-phosphoric acid with a trace of copper sulfate). Cool, dilute to 35 cc. with water, add 10 drops of 1 per cent gum ghatti solution, and make up to 50 cc. with Nessler's reagent, according to the usual procedure for determining non-protein nitrogen by direct Nesslerization. Set up two ammonium sulfate standards, containing respectively 0.09 and 0.15 mg. of nitrogen. The protein content is obtained by the formula

$$P = \frac{100S}{VU} \times N \times 6.25 = \frac{625\,SN}{VU}$$

where P represents milligram protein per 100 cc. spinal fluid, V the volume of spinal fluid taken for analysis, S the reading of the standard, U the reading of the unknown, and N the milligrams of nitrogen in the standard used.

Albumin-globulin ratio in blood serum. This determination most commonly

involves the analysis for total protein, precipitation of globulin by means of 22.5 per cent sodium sulfate solution, and determination of the albumin remaining in solution. The globulin content is usually calculated as the difference between the total protein and the albumin fraction. The sodium sulfate solution used must be exactly neutral. If it is acid, some of the albumin is precipitated with the globulin; if alkaline, not all of the globulin is precipitated. One or two drops of a dilute solution of phenol red may be added to the sodium sulfate reagent solution, and sodium hydroxide or sulfuric acid added until a very faint pink color is visible, when the reaction will be the proper one.

It has been shown[8] that when the mixture of precipitated globulin and albumin solution is poured upon a filter paper to separate them, the first portion of the filtrate contains too little albumin, due to adsorption upon the filter paper. Furthermore, the first portion of filtrate may not be entirely clear. Therefore a small filter paper should be used, the first portion of the filtrate should be discarded, and the last part to pass through the filter should be taken for albumin determination.

Cholesterol. Work in the last few years indicates that the cholesterol content of corpuscles remains fairly constant in pathological conditions, and that the significant variations occur in the plasma. Hence it is usually more valuable to the physician to have the determination run on serum rather than on whole blood. Extraction may be secured by drying the serum on fat-free filter paper and extracting with boiling chloroform under a reflux condenser, or by using the alcohol-ether extraction method of Bloor.

In a small laboratory, the most satisfactory reaction for determining the cholesterol extracted is the Liebermann-Burchard color reaction, in which are employed acetic anhydride plus sulfuric acid.

It has been shown during the last few years that the percentage of cholesterol in the esterified form, the cholesterol ester content, is a more valuable index of changes from the normal cholesterol metabolism than is the total amount in the serum. It is likely, therefore, that there will be an increase in requests for the determination of cholesterol esters. Some of the methods devised for the separate determination of free and esterified forms of cholesterol are beyond the technical capacity of the average medical technician, as well as requiring expensive equipment not ordinarily found in a hospital laboratory. Most well-trained technicians will not, however, encounter much difficulty in using the method for cholesterol ester determination devised by Bloor and Knudson.[9]

Carbon monoxide detection. While quantitative determination of the amount of carbon monoxide hemoglobin present in blood lies primarily in the field of the toxicologist, the medical technician can easily determine the presence or absence of this gas. It must be remembered that if a person exposed to illuminating gas or to automobile exhaust fumes survives his experience, and is removed from exposure to the poison, all of the carbon monoxide disappears from his blood in the course of a few hours. Oxalated blood samples must therefore be obtained promptly upon admission to the hospital, and should be

kept stoppered tightly until the time of the determination. The easiest and most reliable simple method to use is the tannic acid-pyrogallol method devised by Sayers and Yant,[10] and described in Peters and Van Slyke as "an approximate colorimetric method." For qualitative detection it is not necessary to make up a series of standards. Grind together in a mortar equal parts by weight of tannic acid and pyrogallol. In a test tube dilute 0.1 cc. of blood to 2.0 cc. with water. To this add about 40 mg. of the tannic acid-pyrogallol mixture. Treat some known normal blood similarly, and compare the two tubes after 30 minutes. If carbon monoxide is present, the suspected mixture will have a rose to red tint.

Sulfanilamide. This important drug has proved its worth in the treatment of certain diseases. For maximum effectiveness and safety its use must in many cases be checked by determination of its concentration in the blood, inasmuch as the latter depends not only upon the dosage given, but also upon the rate of excretion by the kidneys. This rate in turn varies with the functional efficiency of the kidneys. Fortunately, the determination of sulfanilamide is not difficult, and adequate directions are available in Marshall's articles on the subject.[11]

Thiocyanate in blood plasma. The experience in several clinics seems to be that when the concentration of thiocyanate in the blood is properly controlled by chemical analysis, sodium thiocyanate may be of value in the treatment of certain types of hypertension, or high blood pressure. The method for determination of thiocyanate is simple.[12] Most laboratories have a standard solution of thiocyanate that is used in chloride titrations. This can be diluted with care so that further standardization is not necessary, if one is willing to do a little calculating.

Vitamins. Determination of vitamin C in urine is already feasible.[13] Its titration in blood, accurately, is beyond the capabilities of the average medical technician. We can look forward, however, to simpler and more reliable techniques for determination of this vitamin, and to the working out of methods for other vitamins.

In view of the rapid expansion of knowledge in all fields of medicine, it seems that physicians will have to depend to a greater degree than ever before upon laboratory findings. While waves of enthusiasm and sometimes subsequent disappointment over new discoveries cause a flux and ebb in demand for certain determinations, the amount of routine, essential laboratory work that is necessary for accurate diagnosis, prognosis, and treatment continues to increase.

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TECHNIC OF VENIPUNCTURE*

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Venipuncture, for diagnostic or therapeutic purposes, should be and usually is a very simple procedure. But it is not always skillfully done, and is sometimes performed in a most blundering manner. Perhaps the very fact that it is considered a minor procedure is the reason that men with perfect technic for difficult work will at times make quite a bungle of venipuncture. If one feels that he or she is about perfect in this bit of technic, let him consider that the following are evidences of major or minor lapses from perfection:

1. Poking about in various directions in the tissues before entering the vein.

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2. Passing through a vein.
3. Making more than one attempt before success.
4. Losing blood in the tissues (ecchymoses).
5. The escape of injected substances outside the vein.
6. Puncture of an artery instead of a vein.
7. Cutting down on a vein. This is the resort of the desperate.

It is a curious fact that patients may forget the agonies of a major operation and the tedium of a protracted stay in the hospital to remember the painful and unskillful attempts of the interne at venipuncture. In a large city hospital, at least 25,000 venipunctures for various purposes are done in a year, and so on a quantitative basis alone, this procedure deserves some respect and study.

Equipment. A large hospital service demands many and varied needles for sundry purposes and technicians should familiarize themselves with the features and particularly the sizes of needles so as to recognize them at a glance.

For taking blood, one may use 4 cm. shaft standard needles with or without syringe. The length should be such as to allow a definite margin between the hub and the point of skin entry. The needles are preferably rust-resisting or rustless steel, the former taking a keener point, the latter more flexible and resistant to breakage. The size should be 18 or 19, 18 when larger amounts of blood are wanted and more quickly, 19 in routine work as when small amounts are wanted for Wassermann, Kahn or blood chemistry tests. Where many needles are used, the type furnished by the states of New York and New Jersey for test purposes is handy and cheap. It is a 19 needle for use without a syringe, the hub or milled holder placed in the middle of the 5 cm. shaft, permitting the proximal end to rest in the test tube. For taking blood, we are quite partial to the Graeser needle, originally designed for arsphenamine injections. Smaller needles than 19 are not desirable for taking blood, even with syringe assistance. In taking such large amounts of blood as are indicated in transfusions or in bleeding hypertensives, special needles are employed like the Lindemann, Lewisohn, Unger, Kaliski or Fordyce types. The Unger is a double cannula and

the Lindemann a triple cannula model. The Lewisohn is an ordinary large needle plus a shield. Most of these special makes have a relatively large shield or flange at the hub which makes for easier and more accurate handling. They are made in size from 13 to 18, the larger for taking blood and the smaller for administration purposes. We have found it preferable to use, instead of the double or triple cannula type, a trocar with a closely fitting cannula in three sizes, 11, 12, and 13 and with a shield or flange of such a size that the whole may be dropped into and kept in an ordinary test tube of 1.5 cm. diameter. This type is easier to clean and keep so, and the vein once entered, there is no danger of subsequent puncture of the walls after the trocar has been removed. Employ size 13 for taking amounts of 250 cc. or less of blood or when the veins are small. With sizes 11 or 12, 500 cc. of blood may be obtained in 2 to 5 minutes.

In venipuncture for the purpose of giving fluids or medicaments, the needle-syringe or the needle-burette combination is employed. In general, the needle used is smaller than for taking blood and begins with a 25 or hypodermic size. For burette work, as with citrate transfusions or glucose or arsphenamine injections, the Graeser needle with the useful finger ring atop of the hub is very convenient for easy entry of the vein. Some still prefer the Schreiber, the first special arsphenamine needle. Use an 18 needle routinely, a 19 or 20, exceptionally, for transfusions: use a 20 or 21 or even smaller needle for arsphenamine: and vary the size of the needle with glucose, depending on the concentration, the higher the percentage of sugar the larger the needle, though the maximum size should seldom be over 19. Giving saline with a burette only requires a 21, 20 or 19 needle.

When injecting with syringe and needle, use the smallest syringe which is adequate as the larger 20, 50 and 100 cc. sizes are much more difficult to control both as to the steadiness of the inserted needle and the injection of the fluid. Thin fluids like neoarsphenamine, mapharsen, tryparsimide or calcium chloride may be injected slowly with a 10 cc. syringe and very small needles, 25 to 22, and cause the patient practically no

pain. Glucose 50 per cent is so heavy and thick that a larger needle like an 18 should be used. Substances employed by the roentgenologist, like iopyracyl, also require a needle no smaller than a 20. Injections of sticky antiserums are much more satisfactorily done with an 18 needle than the small needles furnished with trade outfits.

It goes without saying that the needle should be very sharp and kept so. See that the point is not bent or feathered. The bevel may be varied for different purposes: it averages from 3 to 4 mm. and is preferably not longer. A short bevel, scarcely 2 mm. may be desirable as in puncture of the longitudinal sinus. The needle is usually inserted with the bevel up. Bullowa suggests inserting with the bevel down for easier puncture and less likelihood of piercing the distal wall. Needles, like the Graeser, may be sharpened this way if desired.

Preparation. Needles are conveniently prepared as follows. (1) Rinse needle with syringe. (2) Wash inside and out with a sand soap, rinse thoroughly with water, place in alcohol for a short time, dry, wrap in a small piece of gauze or muslin and then in paper. (3) Tie and label with type of needle, size and date. (4) Sterilise in hot air box up to 160°C. and let cool slowly. Needles keep clean this way for all practical purposes for weeks and may be safely used in venous puncture. Instead of this package, they may be put singly or in numbers in test tubes padded in the bottom with cotton. When employed for blood culture, it is well to resterilise them just before use. Needles and syringes may be prepared this way and wrapped up together in gauze and paper.

The site for puncture is wiped with alcohol soaked cotton pledgets or swabbed with 3.5 per cent tincture of iodine. Judging from cultural results after such cleansing, iodine is more efficient. But the sterilisation is never 100 per cent and results are quite satisfactory after either method in that infections from venipuncture are almost unknown in hospital work. We prefer the less troublesome alcohol. In taking blood for cultures, it is good routine to use the alcohol a little longer and then introduce the needle while the arm is still wet. The absence of con-

tamination in the various media is proof that the method is adequate. Local anaesthesia is neither necessary nor desirable.

Choice of Veins. The vein employed in the vast majority of cases is one of the antecubital veins at the bend of the elbow, the median basilic, basilic or cephalic. Regardless of the name, pick a suitable, large vein, but not the best if injections are likely to follow, as the best vessels should be saved for this more difficult procedure. The value of a transfusion donor is almost in proportion to the size and tension of these elbow veins. Failing in elbow veins, the next choice is usually the cephalic vein at the wrist on the thumb side. To make this adequately prominent, turn the half-closed hand as far as possible in the direction of the little finger while compressing the forearm. The median vein on the anterior surface of the wrist, though small, is sometimes useful, as are also the dorsal metacarpal veins on the back of the hand. The walls of these veins, with the exception of the cephalic, are very thin, especially in children, and extra care must be exercised to avoid puncture after entry. The dorsal veins of the foot are rarely used but occasionally serve. The lack of a straight course in varicose veins in the leg makes them unsuitable, though they may seem large. The external jugular is employed mostly in infants in whom the longitudinal sinus is also available. The veins below the elbow and above the wrist are not desirable though they may appear prominent. Neither are those in the arm above the elbow.

The elbow veins are used commonly and indifferently for taking blood or giving injections. The veins of the wrist, hand and leg are used almost exclusively for injection purposes and only occasionally for removing blood samples. The jugular is seldom used at all, then mostly in children, and nearly always for giving and not for taking purposes. The longitudinal sinus should be punctured exclusively for removal of blood and never for injection.

Technic of Venipuncture. In taking blood for any purpose, it is recommended that, where possible, a needle alone be used. This because it is simpler and very much more economical in time (cleaning) and cost of material. Where great numbers of

blood samples are taken, as in a large hospital, the additional cost of syringes and the hazard of breakage is almost prohibitive. The awkwardness of handling the standard syringe needle by the hub alone is soon overcome. And with the New York Board of Health needle and especially the Graeser needle, the handling is easy. Even with blood cultures, the needle suffices, allowing the blood to fill a sterile test tube. Proper amounts may then be apportioned with a pipet to various media with equal or greater accuracy than by syringe allotment. In sedimentation tests, the small syringe needle combination is, of course, used. Taking blood with the needle minus the syringe applies practically exclusively to the elbow veins, but this covers the large majority of blood withdrawals. When blood is removed from the wrist, hand, foot or jugular veins or the longitudinal sinus, it is done much more conveniently with needle and syringe.

The technic of *withdrawal* of blood in routine cases, as in tapping the elbow vein, is as follows. Use as little excess apparatus as possible: one will naturally adopt this line when there are a hundred or more venipunctures to be done in a day. Have alcohol, cotton, bandage and tubes ready and the needle package unwrapped or unplugged. Appraise the patient. If he looks or talks like a fainter, have him prone. Women, as a rule, do not faint. If a series of punctures is being done, as in a syphilis clinic, an assistant will save time in the end. Bare the elbow and see that in a man a rolled-up undershirt, or in a woman a dress sleeve, is not tight over the upper arm. Have a good light. This is as important in venipuncture as it is unimportant in spinal puncture. The arm is extended over the edge of the bed or carrier when lying down, or the desk or table when sitting up. The elbow is in a state of hyperextension for the best result. By sight and feel, pick out the most likely vein. Swab the site with alcohol and dry it. Have the patient compress his own arm tightly and tell him to keep his whole arm stiff and straight and make a fist. All this diverts his attention somewhat from the needle prick. Some prefer a tourniquet in the form of a flat rubber band or a piece of rubber tubing or less preferably a

section of bandage. Or an assistant may hold the arm. A blood pressure apparatus may be used in bleeding for transfusion. If the vein does not stand out tensely, snap it with the finger or vary the pressure. Handle the needle by the hub only, never by the shaft. Use a 19 needle for 15 cc. of blood or less and an 18 needle for more. Hold the needle hub by the thumb and first finger of one hand and the receiving tube with the other hand all in a line, the hub of the needle resting on the lip of the tube. Fixing the skin and vein with the free fourth and fifth fingers of the hand holding the tube, advance the needle and tube as a unit parallel to the long axis of the vein, the needle bevel up (or down) and the needle only at a slight angle from the arm surface. Push through the taut skin and then enter the vein by raising the needle butt a little. Do not stop at the very entrance of the vein but introduce the needle at least a centimeter. The flow of blood indicates successful entry. If the needle has gone through the vein, withdraw it slowly until the vessel is reentered. Such an accident is undesirable but not fatal to this procedure as it may be in giving intravenous injections. Vary the pressure on the arm by the patient, assistant or tourniquet to get the maximum flow. If the sample is for both Wassermann and blood chemistry tests, a large amount may be taken and the latter half poured into the tube containing an anticoagulant. Or better, take a Wassermann sample first, release the pressure above, have the patient open his fist and with the needle in place, simply switch to the second tube with the anticoagulant. This can be done without losing a drop of blood. When sufficient blood is taken, release all pressure, withdraw the needle and tube as one with one hand, holding a pledget of dry cotton tightly over the puncture site with the other. When bleeding is checked, leave a fragment of dry cotton in place and apply a light bandage with the arm flexed. Instruct the patient to remove the bandage in two or three hours, thus avoiding unpleasant constriction.

When it appears impossible, by estimate or trial, to get blood from elbow veins, try other veins suggested, notably those of the wrist or hand, but in such instances, attach a syringe to the

needle, giving the advantage of a lever and making the puncture easier in a more difficult situation. The skin of the wrist and hands is tougher and entry is more difficult and apt to be jerky unless well controlled. The syringe gives this control and aids blood extraction from small veins. Use an 18 or 19 needle, not smaller, even though the veins are small. Various postural and pressure maneuvers are often necessary to bring out these veins. As soon as the blood is taken, expel it from the syringe and disassemble the syringe or rinse it at once with water.

Children are a special problem, but it is a pleasant surprise to find how frequently children and even infants have suitable veins. From 2 to 7 years of age, veins are almost invariably adequate: from 1 to 2 they often suffice. Under one year, and especially in the first three months, venipuncture is admittedly difficult. In infants and children, withdraw blood with syringe and needle, not with the needle alone. Assistance is needed in keeping the arm hyperextended and quiet, and the jugular may have to be used. In infants with the anterior fontanelle open, blood is easily obtained from the longitudinal sinus, using the posterior angle of the fontanelle as a guide and, with the head held firmly, sticking strictly to the middle line. Use in this instance an 18 needle with a bit of solder 5 mm. from the short beveled point to guard against too deep puncture. Enter the needle almost but not quite at a right angle. The blood enters the syringe with ease when the sinus is punctured. One need not be apprehensive in doing venipuncture in hemophiliacs as they seldom give trouble by subsequent bleeding.

Difficulties and Failures. Do not discuss your difficulties before the patient. Good technique prevents troubles. Test the patency of the needle before using. Make the first entry low in a useful vein, so that if a second puncture is required, it may be made higher up. See that the vein is taut before puncturing. Allowing the vein to become lax accounts for many failures. This is most often due to the patient flexing his forearm ever so slightly—guard against this. In loose tissues, the vein tends to double before the push of the needle when the vessel is not held taut. If no blood appears when you believe you have pierced

the vein with a patent needle, withdraw it slightly to see if you have passed through. If withdrawal produces no blood, again attempt puncture without completely withdrawal of the needle. Verify the position of the vein and the needle point with your finger. If failure follows further attempts, try another vein. If the blood does not appear immediately when using a small needle and a small vein, especially when the condition of the patient is poor, wait a second or two before deciding the vein is not entered—a drop may slowly appear. Do not confuse this, however, with blood appearing from a leak outside the vein: this is also apt to come slowly. If blood appears at once but swelling is seen when injection is begun, the needle, especially if long beveled, may be half in and half out of the vein and should be further introduced. If injection, as in transfusions, starts well and then slows up or stops, change the position of the needle slightly within the vein. This trouble may be due to the bevel aspect of the needle being pressed against the wall of the vein. Beware of the prominent, cordy veins of the aged which look inviting but are actually difficult, as without supporting tissue, they dodge and elude the needle. Do not make the mistake of passing through a vein into an artery. The speed with which blood appears is alarming but fortunately usually not serious as bleeding is checked without much difficulty after the needle is withdrawn. Do not puncture the hematoma of a previous day; such a site is often deceiving as the blood may collect around the vein to give the impression of a good vessel. Carefully avoid thrombosed veins.

Sequelae. Serious or even disconcerting results from venipuncture are few. Ecchymoses and usually hematomas take care of themselves. In leukemias, especially the acute types, venipuncture may be followed by a swollen, slightly hemorrhagic area about the puncture site. This lesion is quite characteristic but seems to do no harm.

Infections following venipuncture are extraordinarily rare. This, of course, does not refer to non-professional punctures as in heroin addicts. But it is testimony to the harmlessness of the procedure that many thousands of punctures may be done in a hospital in a year without an instance of infection.

SUMMARY

Venipuncture is one of the most commonly practiced procedures in modern medicine and is largely allotted to internes and technicians. Its performance should not be haphazard but reasoned, scientific, and skillful so as to react favorably on the performer and the institution.

THE TECHNIQUE AND DIAGNOSTIC VALUE OF THE AGGLUTINATION TEST*

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The agglutination test has its widest application in the laboratory diagnosis of those diseases caused by the Gram-negative bacilli. Its diagnostic value depends, however, upon a thorough understanding of the antigenic composition of the bacteria being used. It may be well, then, to review briefly the subject of bacterial antigens using as an example the typhoid bacillus.

As early as 1903 Smith and Reagh found that motile organisms contained two different antigens, one in the flagella and one in the body of the cell itself. Thus antisera produced by injecting the whole organism contained two antibodies, one reacting with the flagella and the other specific for the body or somatic part of the bacterium. This fundamental observation remained unnoticed until 1917 when Weil and Felix re-discovered the phenomenon while working with the X-19 strain of *B. proteus*. To the flagellar antigen they gave the term H (derived from the German word, Hauch, meaning film); the somatic antigen they designated as O (ohne hauch).

Organisms possessing intact flagella behave as though only that antigen were present. This phenomenon is easily understood when one recalls that most bacterial antigens are prepared with formalinized saline. Formalin is, of course, a fixing agent, which thereby stiffens the flagella. Even if the somatic antigen

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at the surface of the cell were prepared for agglutination, the clumping of the organisms would be prevented by the presence of their rigid flagella. Likewise, when an antigen prepared from motile organisms is agglutinated by antibody and electrolyte, the formalin-fixed flagella prevent the organisms from coming into close contact with each other. The result is a loose fluffy sediment. In the absence of flagella, however, the cell bodies are clumped to form small dense granules which are broken up with difficulty.

The H and O antigens differ in their degree of specificity. In general, the H antigen is specific for the species or type, while the O antigen reacts with antisera derived from other members of the same group of bacteria. For instance, O typhoid antigen may be agglutinated by *B. paratyphosus B* immune serum but H typhoid antigen never is. This does not mean that the O antigens of *B. typhosus* and *B. paratyphosus B* are identical. It does mean, however, that O antigen is actually composed of several different constituents and that related species such as *B. typhosus* and *B. paratyphosus B* may have one or more of these antigens in common. It must be borne in mind that the species producing enteric fever such as *B. typhosus*, *B. paratyphosus A*, *B. paratyphosus B* and the members of the Para B or Salmonella group show such relationships. Reactions occurring between different members of the same group are termed cross agglutination.

To add confusion to an already complex subject, not all H antigens are specific. That of *B. typhosus* is, but *B. paratyphosus B* H antigen may or may not be species specific. The latter antigen is termed diphasic. It is no wonder, then, that the serological diagnosis of enteric fever is difficult and that the interpretation of results must be made with care.

ENTERIC FEVER

Let us turn now to the practical applications of these facts. It has already been stated that H antigen may be prepared by the use of formalin. Either a young, actively-motile and smooth broth culture or washings from beef infusion agar plates may be used. The latter is to be preferred. The O antigen is best

obtained by using a stable, smooth and non-motile variant such as the O-901 strain of *B. typhosus* which is then treated with alcohol. If such a culture is not available or can not be obtained from the state Health Laboratory, a motile strain may be employed since alcohol destroys the flagellar antigen anyway. The preparation of both of these antigens is described in detail in the newer texts on laboratory technique and is given in detail in the American Public Health Association Year Book, 1935–1936, pages 144–158.

The technique of the agglutination test is a simple one. By serial dilution various concentrations of the patient's serum are placed in a row of tubes containing diluted antigen. The turbidity may vary within limits but must be such that by gentle shaking the swirling of the cells is plainly visible. The greater the turbidity the less delicate the test. The optimum incubation temperature appears to be either 50°C. for 18 or 20 hours, or 55°C. for 2 to 4 hours and ice-box storage over night. The results are graded from 1+ to a maximum of 4+ depending upon the clearness of the supernatant fluid. The highest dilution of serum producing a 3+ or 4+ agglutination constitutes the titre.

If a titre of 1:80 or higher is obtained it is generally considered as diagnostic. Unfortunately, however, this is not always true. Typhoid vaccine, for instance, sometimes results in the production of agglutinins to a titre higher than 1:80. The rise in H and O agglutinins, however, is not equal, since the H agglutinin predominates throughout. In contrast, an actual infection with *B. typhosus* results first in the appearance of O agglutinin and then a subsequent rise in the H. There exists, then, a quantitative difference between the response of agglutinins to vaccination and to the natural infection. In spite of vaccination an O antigen titre of 1:200 or higher indicates active infection. Likewise, a very high H titre (1:1000) is extremely significant.

It has been stated that a titre of 1:80 is generally considered diagnostic. Reactions occurring only in lower dilutions are common and frequently indicate, in uninoculated individuals, the presence of so-called "natural" agglutinins. Their origin is uncertain but probably due to sub-clinical infection from time

to time by the same or related organisms in the typhoid-paratyphoid group. The diagnostic titre has to be, therefore, above the "normal" agglutinin level in any given locality.

It is not rare to experience a sudden sharp rise in typhoid-paratyphoid agglutinin resulting from a non-enteric febrile disease if the patient has previously been vaccinated or has had enteric fever. This is called an anamnestic or memory reaction. The mechanism is not clearly understood but may well be due to infection by a microorganism containing an antigen which is also present in the members of the typhoid or paratyphoid group. In a similar manner, an individual who has had typhoid fever and who at the time shows only low agglutinin content in the serum may, upon becoming infected with one of the paratyphoids, show a sudden and more rapid rise in typhoid agglutinins than in homologous paratyphoid antibodies.

Even when the theoretical considerations above are carefully weighed, it is at times difficult to interpret agglutination results. In such instances repeat tests are strongly recommended. A gradual rise in titre, especially when both the H and O titres rise, indicates active infection. By the same token, a negative test is not without significance since, if a repeat test one week later shows slight agglutination, there is every indication that the serum represents an early enteric infection.

Finally at what stage of the disease do agglutinins begin to appear? The most reliable information available is that regarding typhoid fever. Thirty-five per cent of cases are positive at the end of the first week; 65 per cent at the end of the second and 80 per cent at the end of the third week. By the fifth week 95 per cent of all typhoid sera will be positive. In the light of this information it is clear why a negative test on a clinically-suspicious patient must be repeated together with stool and blood cultures.

Enteric fever is not a bacteriologic entity but may be produced by at least twelve different members of the typhoid-paratyphoid group. How many of these types must be represented in order to avoid the possibility of missing a positive serum? Fortunately, by the judicious use of H and O antigens

a large number of tests is not necessary. For instance, an H typhoid test will detect specific H typhoid agglutinins indicating either vaccination or active infection. An O typhoid will provide the most sensitive index of actual infection but is subject to cross agglutination with other members of the typhoid-paratyphoid group. The addition of a Para B test using the H antigen will suffice since it is commonly found that H antigens prepared from this organism are sufficiently diphasic or non-specific to react with most of the Para B group. The routine use of antigens prepared from other members of the Para B group is not essential or advisable since it is of little clinical value which member of the Salmonella is producing the infection. Para A is of such infrequent occurrence in this country that inclusion of this organism appears unnecessary.

For the sake of completeness the Vi antigen must be mentioned. Felix and Pitt in 1934 reported that certain strains of *B. typhosus* are not agglutinated by O typhoid agglutinin and that from these particular cells could be isolated a new antigen. This they called Vi because its presence bore a direct relationship to the virulence of the organism. Its importance in routine laboratory tests is not established, however, since no case of typhoid fever develops Vi antibody without being accompanied by either H or O agglutinin.

The rapid microscopic test either with living or killed culture should be used only as an accessory procedure. This method does not possess the same degree of reliability as the macroscopic test but may be of distinct value as a preliminary method when large numbers of routine sera must be handled.

In conclusion, the accurate laboratory diagnosis of enteric fever by agglutination depends upon:

(1) Careful selection of a smooth motile strain for the preparation of H antigen.

(2) Use of O typhoid antigen best prepared from a smooth non-motile culture.

(3) A Para B antigen which is not specific.

(4) A knowledge of the effect of vaccination upon antibody production and of the level of natural agglutinins.

(5) The stage of the disease when the serum was collected.
(6) A repeat test in case of doubt.

BRUCELLOSIS

Undulant fever is caused by a group of Gram-negative bacilli known as the Brucella. There are three types within the group, but as far as agglutination is concerned it makes little difference, since a smooth culture of any type is easily agglutinated by any Brucella serum. Here the agglutination test is simple, since *Br. abortus* is non-motile and therefore contains only the O antigens. It is our custom to employ this test on all routine sera submitted. It should be emphasized, however, that approximately 10 per cent of infected individuals fail to produce detectable agglutinin. The diagnostic titre is generally considered as 1:50.

TYPHUS FEVER

Although caused by an organism not readily cultivated (Rickettsia prowazeki), the disease may be detected by the use of a certain strain of *B. proteus* (X-19). It was while working with this organism that Weil and Felix discovered their H and O antigens. This apparent anomoly has been shown to be due to a common O antigen existing in both microorganisms. As a result a non-motile strain of *B. proteus* X-19 should be used. If it is non-motile, a fresh living culture is an excellent antigen. Non-specific reactions are common so that a titre below 1:320 should be interpreted with caution.

TULAREMIA

Tularemia is a disease of lower animals, transmissible to man and occurring most commonly in the west and south. The organism is cultivated with difficulty but the reaction is very specific. A titre of 1:80 may be considered diagnostic but a *Br. abortus* agglutination test should be run at the same time since these two organisms occasionally cross-agglutinate.

AN EVALUATION OF METHODS FOR GASTRIC ANALYSIS*

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By means of gastric analysis we are able to study gastric work. The whole purpose of gastric work is to reduce food to chyme. We define chyme as that thick, grayish liquid mass to which food is converted by the process of gastric digestion. There are two mechanisms involved in gastric digestion, secretory and motor. The secretory mechanism is concerned with the elaboration of hydrochloric acid and the proferments of rennin and pepsin. The motor mechanism is concerned with churning the food about and bringing it into intimate contact with the gastric mucosa. Finally, after a variable period of time, depending on the type of food ingested, the motor act is completed when the food is propelled into the duodenum. Any disturbance in either of these two mechanisms whether on the side of hyper or hypoactivity may result in gastric symptomatology because of attendant gastric dysfunction.

The question is often raised, why should one bother with gastric analysis when the patient can be x-rayed? The answer lies in the fact that the information gained from the gastric analysis is quite different from that obtained from x-ray. Gastric analysis is a study of gastric physiology, normal or abnormal; x-ray diagnosis is mostly an anatomic one, giving information as to the shape, position, contour, type, presence or absence of growth or ulcer, etc. The earliest lesion of organic gastric disease may begin in the mucosa and at this early stage x-ray examination is of no value because, to obtain a positive diagnosis by x-ray, there must be a definite defect or indirect evidence in the anatomic contour of the organ. In that developmental stage between the inception of the organic lesion and the point at which a definite defect occurs the x-ray usually fails us and the report will come back "no organic abnormality noted." Yet by gastric analysis we may obtain information that a lesion

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is present because the presence of pathology interferes with gastric physiology and this will manifest itself in deviations from the normal gastric findings Likewise gastric analysis is of value in observing dysfunction due to the functional gastric disorders.

We prepare our patient for study by having him take a motor meal twelve hours before he reports to us. At 9 P.M. of the preceding evening we have him take a meat sandwich, 20 raisins and a glass of water or tea. He is not to eat or drink anything till he reports at 9 o'clock of the following morning. We also order him not to brush his teeth on going to bed or on arising in the morning. We caution him also against swallowing saliva or any post nasal discharge. Such a motor meal should leave the stomach in 2–3 hours, or at most 5 hours in the slow type of digesting stomach, so that when we extract the fasting gastric contents in the morning the latter will give us a picture of mucosal activity in the interdigestive or rest phase, and indirectly that of the motor mechanism. We caution against brushing the teeth so as to prevent positive occult blood findings due to trauma of the gums. The swallowing of saliva, because of its alkaline reaction, will lower the fasting gastric acidity estimation. The presence of swallowed post nasal discharge interferes mechanically with the microscopic study. When the patient reports in the morning he swallows the stomach tube for a distance of about 55 cm. which in the average case will place the tip of the tube in the antrum. The entire fasting gastric residuum is removed by gentle suction and this examined for the following facts. A proper evaluation of the data so obtained will yield us information of considerable value.

FASTING GASTRIC RESIDUUM

1. *Amount*: Normally we can extract from a few to 50 cc. Lyon gives 80 cc. as a high normal and Rehfuss states that 100 cc. may be normal. Here in the clinic we regard 50 cc. as a high normal; anything above this amount is due either to retention or hypersecretion. The microscopic picture will help us decide which it is. Retention may be due to obstruction of the pylorus, either intra or extragastric; to pylorospasm or to

weakness of the gastric musculature. Hypersecretion results because of reflex stimulation of the gastric mucosa and is usually due to a lesion outside of the stomach as in duodenal ulcer.

2. *Color and Odor*: The color is normally pearly gray; any other color is an abnormality; exception—gagging and regurgitation. The presence of bile or pus will alter this color. The odor is pleasantly aromatic; any other odor is abnormal.

3. *Consistency and Sediment*: The consistency is that of a thin mucoid fluid. The sediment comprises 5 per cent of the gastric filtrate. Sediment consists of exfoliated epithelium, few leukocytes and a few bacteria that are in transit. Any increase above the 5 per cent in sediment signifies that we are dealing with an increased amount of the above element.

4. *Food Retention*: Gross and Microscopic—Gross food retention implies that we are dealing with an advanced organic lesion at the pylorus. Such a lesion may be either intragastric—as ulcer at the pylorus with stenosis, or an acute ulcer with inflammatory edema or a carcinoma at the pylorus. The lesion may be extragastric due to adhesions about the pylorus or to the pressure of a tumor mass upon the pylorus, for example a carcinoma of the pancreas. Exceptionally a very marked degree of pylorospasm or rarely extreme ptosis with ectasis may also cause gross food retention.

Twelve hour microscopic food retention is also abnormal. It may be due to an early stage of one of the above lesions but more frequently we find it due to pylorospasm either reflex or functional, or to weakness of the gastric musculature as in visceroptosis with atony.

5. *Biliary Regurgitations*: We regard the presence of bile in the fasting gastric contents as abnormal except under two circumstances: (1) if the patient gags on taking the tube it is obvious reversed peristalsis may occur in the duodenal segment, and (2) if hyperacidity is present reflux of alkaline duodenal contents may occur in a physiologic attempt to lower the high acid values. If the patient has not gagged on taking the tube, and the fasting gastric acidity is not abnormally high, the presence of gross bile signifies to us that we are dealing with an extragastric lesion in

the duodenum or gall bladder producing the motor defect of reversed duodenal peristalsis, arteriomesenteric occlusion etc. The presence of bile in the fasting stomach day after day is often a prime cause of morning nausea, of bitter taste and of loss of appetite for breakfast. Biliary regurgitation during the digestive cycle is likewise abnormal except where the gastric acidity is exceptionally high. Bennett and Ryle and Rehfuss claim that biliary regurgitation frequently occurs in normal individuals. This point is not definitely proven.

6. *Gross Blood*: There should be no gross bleeding. When present this suggests peptic ulcer, ruptured esophageal or gastric varices incident to portal cirrhosis, carcinoma, bleeding papilloma, syphilis, or a blood dyscrasia, etc.

7. *Occult Blood*: Occult bleeding is also abnormal if good tube technic is used. It is at times difficult to evaluate the importance of occult blood because it may occur from extragastric sources—as gums, teeth, nasopharynx. We grade our occult blood reactions plus 1, 2, 3 and 4. Plus 1 reaction we regard as possibly due to trauma. If the reaction is stronger we must think of one of the conditions mentioned above under gross blood. Its presence as a rule is less significant of serious disease since it frequently accompanies gastritis or it may be an expression of congestion, or erosion or mucosal ulceration. There is also the possibility that the presence of occult blood in any one fraction of the digesting stomach may be due to an abnormal amount of peristaltic contraction against the tip of the tube with consequent mucous membrane erosion.

8. *Abnormal Amounts of Mucus*: Marked excess of mucus is significant of catarrhal gastritis, achylia and if in great quantity—gastromyorrhoea (rare).

9. *The Degree of Free Hydrochloric Acid and Total Acidity*: The theoretical normal for free hydrochloric acid in the fasting stomach is generally stated to be from 10–25 per cent acidity and for total acidity from 20–40 per cent. There are many factors which may influence the fasting acidity when tested out on any one morning such as indiscrete diet, poor night's sleep, fear of swallowing the tube, nervousness, worry and the like.

10. *Microscopy*: The microscopy of the fasting gastric residuum is of great importance. Its interpretation is of more value when expressed on a quantitative scale based on the percentage of sediment to filtrate. We use unstained fresh spreads and look for the following:

(a) Excessive exfoliation of epithelium: We can recognize under the 'scope, oral, respiratory, esophageal, gastric, duodenal and biliary epithelium. An excessive exfoliation indicates an abnormal rate of cellular death from the region concerned, and local inflammatory changes.

(b) Abnormal increase in pus cells or their nuclear remains suggesting inflammation. We may find such an increase in all cases of gastric ulcer, in gastritis whether of the inflammatory or congestive type and in cancer of the stomach affecting chiefly the fundus.

(c) Abnormally excessive bacterial flora, especially if in colony formation, suggesting infection. Comparison with the type of cells found aids in localization of source, e.g.—in excessive oral epithelium search for oral sepsis, etc.

(d) Abnormal elements as sarcinae, yeast cells, Oppler-Boas bacilli etc. The presence of sarcinae and many yeast cells suggests gastric dilatation with stagnation and fermentation of contents. Oppler-Boas bacilli may be found in subacid or anacid gastric juice associated with retention and stagnation; their presence is suggestive of carcinoma but not pathognomonic.

(e) Microscopic retention of food: We look for remnants of food eaten the night before such as meat fibers, striated or partially digested; starch granules; fruit pulp; fat globules;—indicating some degree of motor delay (pyloric obstruction or advanced atony) or rarely to abnormally deep rugae or crypts.

PREPARATION OF PATHOLOGICAL SPECIMENS IN NATURAL COLOR*

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Since gross pathological specimens are prepared primarily for teaching demonstration it is indicated that they should be preserved with a retention of as many natural characteristics as possible. These characteristics consist of size, shape, color, consistency and—in some instances—odor. It is by these features that gross specimens are identified; therefore, the more of the characteristics preserved in the material the better the method of preparation.

Unfortunately, a perfect method of preservation does not exist at the present time, since all of the available methods exercise more or less of an altering influence on the specimen. In other words, in practically every method of preparing material size is reduced due to the shrinking action of the fixing fluid. Shape is apt to be distorted, unless one takes particular care to maintain this quality during the fixing period. Consistency is usually increased, this being advantageous generally because of the handling which specimen material subsequently undergoes. Odor is eliminated, this being desirable particularly in instances where gangrenous or other foul-smelling materials are being prepared. The most unfortunate change which takes place in preserved specimens is the loss or diminishing effect on natural color. The color of a specimen is important because it is a means by which a more immediate identification of the material is made. To illustrate this, if we take a cross section of an arm or leg we note a striking differentiation between red muscle, yellow fat and white bone. If we thoroughly bleach the same specimen we must depend on shape and consistency in order to tell one structure from another. This fact discloses, in an

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exaggerated manner, the situation which confronts students who attempt to visualize accurately various pathological processes from teaching specimens which possess a shrunken, distorted and colorless condition. It might be said that the reduction in size incurred during preparation is of lesser importance, since topographical relationships are still intact even though size is reduced. But it is to be emphasized that the preservation of correct morphology and natural color are two features which must be retained if one is to accomplish good preparation technique.

The literature contains more than sixty methods by which natural color preservation can supposedly be attained. Few of these have proven of worth, but practically all are based on the same principles, that of retaining or reclaiming the red color of the blood content of the specimen. In general, few of the colors other than the red hues are influenced by modern fixing and preserving fluids; therefore, it is necessary only to maintain the color of the blood content of the specimen to accomplish the best color-preservation which exists at the present time. This is a relatively simple process, but as stated before, the preservation of color alone does not constitute good specimen preparation. More care is probably required to maintain the desired shape of the specimen than is necessary to secure good color preservation.

Regardless of the method selected for preparing specimens certain details common to all methods should be observed. It might be mentioned that accurate preparation methods begin with the pathologist who prosects the body from which the specimens are removed. Many excellent specimens, of potential value for teaching or museum demonstration, have been ruined by haphazard dissection. The success or failure of specimen preparation methods depend somewhat on the freshness of the material. More important are correct interpretation of the technical method selected and accuracy in preparing the necessary fluids and reagents. The ultimate disposition of the specimen should be planned, so that necessary sectioning or dissection may be completed before fixation is begun. In preparing material for temporary preservation a method having

economical features might be indicated, but the element of economy should not be considered where permanency is desired, since any specimen worthy of perpetuation is worth preparing properly.

Of the numerous color preserving methods available, the author prefers that process of Kaiserling which was published in 1897. The original Kaiserling method appeared a year before, and a third method was described in 1899. Many of the textbooks on technical methods give faulty interpretations of these methods, this being the reason for some of the failures experienced by technicians who are being initiated into specimen preparation work. The details of the "1897" method, which the author recommends, and a slight modification of the process are given in the following:

Specimens are fixed in a solution consisting of:

Potassium acetate	30 grams
Potassium nitrate	15 grams
Formalin, full-strength	200 cc.
Water	1,000 cc.

The fixation period requires individual attention for each and every specimen, it being important that the material becomes thoroughly hardened yet not overfixed. Overfixation militates against good preservation of color, and insufficiently fixed material causes disaster when the specimen is finally mounted. Important also are the steps necessary to maintain the desired shape and position of the specimen during this fixing process. This is arranged for in accordance to the character of the specimen. The brain, for example, must be suspended (by a thread attached to the basilar artery) in the fluid, otherwise it may become distorted by resting on the bottom of the fixing container. This same principle must be exercised in preparing other tissues which may flatten by their own weight on the bottom of the jar. To further illustrate the principles of position-fixation, vesicular organs must be distended with as well as immersed in the fixing fluid; cavities and tube-like structures must be given similar preparation so that they remain patent; large organs, such as the liver, should be incised and the incisions packed in order that the fixative may act upon the deeper structures; extremities should be perfused with the fixing fluid, otherwise the skin will develop wrinkles and the epidermis will shed; other skin-covered structures, and specimens enveloped with tough capsules should be injected with syringe and needle; membranous specimens, as opened intestine, need to be tacked in position on cardboard if the distorting action of the fixing fluid is to be avoided. In many instances of preparing large organs it is de-

sirable to cut the material in slabs so that hardening takes place more rapidly and efficiently. In any event, the rule to follow is to obtain complete fixation in as short a time as possible. The time required for fixation varies, of course, with the size and consistency of the specimen. Thin membranous structures may require only a few hours in the fixing solution, while other material may require as many days or even weeks. The progress of fixation may be determined in some measure by the firmness or consistency assumed by the specimen. Another index is the presence of red colored blood in the deeper portions of the material; this denotes incomplete fixation, since hemoglobin should be brown in color when fixation is complete. For all types of material a liberal quantity of the fixative should be used, usually five or more volumes of the solution to a volume of specimen.

With fixation completed the specimen is then ready for the re-development of its color. Kaiserling's method calls for prolonged washing of the material after fixation, but the author has obtained much more brilliant results by modifying this step. Instead, the specimens are rinsed, drained and blotted, then immersed in strong (95 per cent) alcohol where they remain until the red colors develop the desired brilliancy. The time for this step is variable, each specimen requires careful watching. The developing is best observed over a white background. Some specimens require only a few minutes in the alcohol, while others may require an hour or two, or even longer. Overdeveloping will result in a diminishing effect on the color.

The next procedure is an important one. The specimen is to be washed very thoroughly under running tap water, it being necessary to remove all traces of alcohol and the fixing fluid. This washing is extended according to the size and thickness of the material, over-night washing is sufficient for the average-sized specimen, but large organs may require as long as two or three days under running water. If this step is done haphazardly the colors fade when the specimen is finally preserved.

The final preserving fluid, as suggested by Kaiserling (1897), is a solution of:

Potassium acetate	200 grams
Glycerine (water-white)	400 cc.
Distilled water	2,000 cc.

This solution will culture certain types of mould at room temperature, therefore it is necessary to add some antiseptic to prevent this. Some authors suggest small quantities of formalin, but this has a tendency to influence color in time. Phenol should never be included in the preserving fluid, since it gradually causes the blood to turn brown in color. Arsenic and chloral hydrate have been used with some success, but a crystal or two of thymol left floating on top of the fluid seems to be the most practical mould preventive. Sufficient fluid to cover the specimen is all that is necessary to insure preservation. Specimens intended for mounting should be transferred to fresh preserving fluid before the jar is sealed.

The method of Kaiserling has been extensively modified by other authors, most frequently by the addition of illuminating gas or chloral hydrate to the fixing fluid. Such modifications eliminated the need of alcohol, since the colors are not lost during fixation. In general, however, these modifications are of doubtful advantage.

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ANAEROBIC CULTIVATION OF BACTERIA AS A ROUTINE LABORATORY PROCEDURE*

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It is not an unusual experience in the laboratory to observe by direct smear organisms which subsequently fail to grow by the ordinary cultural methods. Such a situation is especially disappointing when the cultures remain sterile because, in the face of microscopic evidence to the contrary, an essentially negative report must be submitted to the clinician.

Let us pause for a moment to consider the probable reasons why these bacteria refuse to grow. It is possible, for instance, that they were dead, or that the inoculum may have been too small. In some instances, this is apparently true. Nevertheless, it is more likely that either the media did not contain

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the proper ingredients or the bacteria were prevented from growing by the presence of oxygen. A few preliminary trials with various types of media convinced us that this factor was not the fundamental one. Furthermore, recent reports by Colebrook[1] in England and by McDonald, Henthorne and Thompson[2] at the Mayo Clinic, illustrate the growing belief that strictly anaerobic streptococci are frequently present in infective processes and usually overlooked. Therefore, it was decided to make duplicate aerobic and anaerobic cultures upon a large series of consecutive routine specimens.

Many different methods have been proposed for the anaerobic cultivation of bacteria. A method which is to be practical in a clinical laboratory, however, must accomodate a large number of plates and, of course, produce rapid and efficient anaerobiosis. None of the older procedures does this. For example, the simplest method consists of boiling a tube containing a tall column of broth or agar and sealing the tube with paraffin or layering the surface of the medium with oil. This is an entirely mechanical process; the boiling simply removes the dissolved oxygen and the sealing operation prevents the re-entry of more air. There are several objections to this type of technique. First, strictly anaerobic conditions are not attained because some residual oxygen remains in the tube. Secondly, each tube must be treated separately. Thirdly, isolation and identification of the various organisms is difficult or impossible. Only by growing the culture on the surface of solid media can positive identification be made.

A widely-used method suitable to surface cultivation is a modification of that devised some years ago by Buchner. The effectiveness depended upon a chemical reaction between acid pyrogallol and sodium hydroxide. After streaking an agar slant, the cotton plug is pushed about one inch below the mouth of the tube, the chemicals added and the tube immediately closed by a rubber stopper. Although effective, this technique has certain disadvantages. If too much chemical is added, seepage through the cotton occurs and chemical disinfection follows. In addition, the process must be repeated for each tube.

McIntosh and Fildes[3] twenty years ago introduced the use of large enclosed jars in which could be placed the plates and tubes. Hydrogen was introduced which, through the action of a heated catalyst, combined with the oxygen. This method was convenient but expensive and accompanied by considerable danger of explosion. The method used throughout this study is based upon the same principle but modified to the extent that the jar is first evacuated and that the catalyst does not require heating. The danger of explosion is thereby removed. The details of this method are given in an article by Weiss and Spaulding.[4]

Approximately nine hundred consecutive routine specimens have been compared by making duplicate aerobic and anaerobic cultures. The total number of cultures is divided into two series. For the first series meat extract blood agar was used; in the second a beef infusion blood agar. The two plates were treated in an identical manner except during incubation.

Our primary objective was to eliminate the incidence of negative cultures in those instances in which the direct smear had revealed organisms. In the first series of nearly five hundred specimens 19 per cent of the cultures were positive only on the anaerobic plate. The figure given above indicates only the absolute presence or absence of growth and does not consider the species recovered. If these anaerobic types are of little significance in human infection, one may well question the value of the additional effort. An analysis of the cultures which were positive only on the anaerobic plate, however, shows that the most frequent organism recovered was the hemolytic streptococcus. Since this species is extremely important in human disease, it is interesting to note that 65 per cent of the hemolytic streptococcus isolations were made only by means of anaerobic plates. The distribution of the anaerobic types was not limited to one source such as the mouth or stool. Among the other species showing the highest incidence of anaerobes were the non-hemolytic streptococcus and the pneumococcus.

It should be emphasized, however, that the great majority of the organisms, which upon primary culture appeared to be

strict anaerobes, became quickly adapted to aerobic cultivation. Indeed, aerobic sub-cultures from the primary anaerobic plate would, as a rule, produce good growth.

At the suggestion of Dr. Casman of the Abington Hospital a second series of approximately 400 specimens was run using, instead of meat extract as a base, a medium prepared from beef infusion and containing 1 per cent Neo-peptone. According to Hedley Wright[5] certain of the most fastidious pathogens are inhibited by oxidized constituents of the peptone. The deleterious influence of these substances can be removed apparently by adding the peptone to the infusion before the heating process. The result is also a lower oxidation-reduction potential.

The purpose of the second series, then, was to determine whether the discrepancies between the aerobic and anaerobic plates could be diminished. In other words, could one by employing a better medium, remove the necessity for making anaerobic cultures? The use of beef infusion medium, prepared according to Wright, reduces the incidence of negative cultures both aerobically and anaerobically. Eighty-seven per cent of the specimens were positive on both plates, and 2 per cent only by anaerobic culture. Nevertheless, 11 per cent of the specimens would still have been reported as sterile without the aid of the anaerobic plate.

Again considering the hemolytic streptococci, the incidence of the anaerobic type, although dropping from 65 per cent to 41 per cent by the use of infusion medium, was significantly high. It is of particular value, therefore, to include an anaerobic culture in the isolation of hemolytic streptococci from clinical sources. Furthermore, the frequent isolation of nonhemolytic streptococci and pneumococci which are strictly anaerobic upon primary culture, as well as the established anaerobic species such as *Cl. welchii*, fusiform bacilli and Bacteroides, justifies, we believe, the routine use of anaerobic cultures. In fact, it is apparent that cultural studies can not be considered complete without them.

The data just presented fail to indicate the superiority of the anaerobic plate in one important respect. It was rather con-

sistently observed that hemolytic streptococci grew better and more rapidly on the anaerobic plate. In our hands this was a valuable aid, since an accurate report could often be made 24 to 48 hours sooner than would otherwise have been possible. Non-hemolytic streptococci and pneumococci behaved in this manner less frequently.

In conclusion, we feel that:

(1) An anaerobic method permitting the use of agar plates is a valuable aid to laboratory diagnosis.

(2) For thorough cultural studies it is indispensable.

(3) An infusion medium prepared according to Wright is recommended.

(4) Anaerobic cultures are especially valuable in the detection of hemolytic streptococci.

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BACTERIOPHAGE TECHNIC*

DOROTHY SAGE

I. METHOD OF ISOLATING BACTERIOPHAGE

a. Emulsify about one gram of the stool in ten cc. of broth.

b. Incubate over night.

c. Centrifuge to throw down the large particles.

d. Filter through a Berkefeld 3 W.

e. Test the filtrate for bacteriophage against B. coli, B. dysenteriae, B. typhosus and staphylococcus.

II. METHOD OF TESTING FILTRATE FOR BACTERIOPHAGE

a. Place five tubes containing 10 cc. broth in rack.

b. To the first four add 0.1 cc. (18–24 hour) broth culture of the test organism.

c. To the first three tubes add 0.5, 1 and 2 cc. respectively of the filtrate to be tested.

d. Tube four is a culture control.

e. Tube five contains 10 cc. broth and 1 cc. of the filtrate to be tested and is a filtrate control for sterility.

f. Incubate at 37°C. until perceptible growth occurs in the control tube (about 4 hours).

g. Observe frequently for lysis.

++++ complete clearing—no sediment
+++ slightly cloudy—no sediment
++ perceptible clearing—some sediment
+ slightly clearer than the culture control

h. Filter the first two tubes through a sterile Berkefeld.

Incubate the third tube over night, a second reading is made and if lysis is not ++++, the test is repeated, using the filtrate after the first incubation. If lysis is complete, a volume of bacteriophage is prepared, using same materials, same quantities, but a greater number of tubes.

III. TESTING AND DISPENSING BACTERIOPHAGE

a. *Bacteriophage should not be issued for treatment unless proven to be lytic for the infecting organism.*

b. Use an 18–24 hour broth culture of the infecting organism.

c. Inoculate tubes containing 10 cc. broth with 0.1 cc. of the culture and 0.5 cc. of the bacteriophage. Also inoculate one culture control tube and one bacteriophage control for sterility.

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d. Incubate tubes at 37°C. until organisms are lysed (usually 4 hours with staphylococcus).

e. Every tube should be less turbid than the culture control which has increased in turbidity.

f. Bacteriophage should be cultured for sterility by incubating 48 hours. None should be used which shows the slightest growth.

g. Bacteriophage which is kept in the refrigerator remains potent for long periods of time.

h. Careful aseptic precautions should be followed in dispensing.

IV. METHOD OF PREPARING STAPHYLOCOCCUS BACTERIOPHAGE AT TEMPLE UNIVERSITY HOSPITAL

a. Inoculate the number of tubes to make the quantity of bacteriophage needed. (At TUH 0.2 cc. lytic bacteriophage and 0.3 cc. 6 hour broth culture.)

b. Incubate at 37°C. until lysed (usually 3–4 hours).

c. Filter through Berkefeld filter and test for sterility.

V. USE AND CARE OF BERKEFELD FILTERS

a. Berkefeld 3W, 5W and 10W are recommended. The test tube containing the filtrate may be removed and replaced by an empty sterile tube. Thus the filter may be used two or three times without contamination.

b. New filters must be cleaned of the chalk by brushing with a finger nail brush free from soap. Forcing water through the filter from the bottom is good.

c. Boil filters 15–20 minutes in distilled water three times. The mantles are washed with soap and water and allowed to drain dry.

d. The filter is reassembled. For small amounts a 15–20 cc. test tube can be used but in preparing a larger volume the 60 cc. tube is used. The air is drawn out of the Erlenmeyer flask by suction and then out of the tube through the second hole and between the two corks producing enough suction for filtration.

e. After being assembled the filters are sterilized in a steam autoclave 1 hour.

f. After use they are dismantled, washed and scrubbed as when new. Boil 30 minutes. The mantles are washed with soap and water and allowed to dry. The filter is re-assembled and distilled water is drawn through by the suction pump to cleanse the interior.

THE PRESERVATION OF BACTERIAL CULTURES. II. SUMMARY OF METHODS*

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The preservation of bacterial cultures for a long or even a short period of time is a problem which confronts nearly every laboratory worker. Several methods have been devised. These will be grouped under two headings. (For fuller details of the various methods see Morton and Pulaski.[2])

I. PRESERVATION OF BACTERIAL CULTURES BY THE PREVENTION OF SLOW DRYING OF THE CULTURES

1. Cultures are often preserved by pouring sealing-wax, paraffin or similar materials over the tops of the cotton stoppers, or, by dipping the cotton stoppers into melted paraffin and then inserting into the culture tubes.

The objections to these methods are the difficulties in removing the materials from the tubes during the cleaning process and the cultures are not protected from contaminations by molds or from dissociating.

2. The tops of the culture tubes may be covered with a rubber cap, tin foil or parafilm†.[3]

The objections to this method are the expense of the materials and the cultures are not protected from contamination by molds.

3. The culture tubes may be sealed off as ampoules in a blast lamp. The objections here are the destruction of culture tubes, the difficulties often encountered in the opening of ampoules and the cultures so preserved readily dissociate.

4. Over-layering the culture with sterile paraffin oil. The method is inexpensive, prevents evaporation of the culture media and dissociation of the micro-organisms and protects the cultures against contamination by molds. The method is applicable to

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† Obtainable from Menasha Products Co., Menasha, Wisconsin.

single colonies or mass cultures. The cultures are always available for subculturing without disturbing the preservation of the stock. Cultures may be maintained in stock under conditions not inducive to aseptic technic.

II. PRESERVATION OF BACTERIAL CULTURES BY THE RAPID DESICCATION OF THE CULTURES

Rapid desiccation appears to be necessary for the survival of the maximum number of organisms. Low temperatures during the drying process appear to be less detrimental to the microorganisms but the optimum range of temperature for each organism, or group of organisms, is not known, quantitatively.

1. Cultures may be deposited on bits of sterile filter paper, glass beads, etc., dried in vacuum over a desiccant and stored in a vacuum according to the technic of Brown.

2. Cultures may be concentrated by centrifugation, the sediment mixed with a small amount of sterile milk, serum, etc., deposited into small sterile tubes and the cultures then frozen and dried in a vacuum over a desiccant. The dried cultures are preserved by sealing the ends of the tubes in the blast lamp or with paraffin or other similar materials, as per the technic of Swift. Paraffin seals are not satisfactory because of seasonal temperature changes.

3. One may employ, for the drying and sealing of the cultures, a specially designed apparatus such as that devised by Elser, Thomas and Steffen or the lyophile apparatus as described by Flosdorf and Mudd or, more recently, the cryo-chem apparatus by the same authors.[1]

To list a few of the advantages of this type of preservation: the organisms are preserved, as far as we know, without changes in their biochemical or immunological properties; the preserved cultures require little space for storage and are in a convenient form for shipping.

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PUBLISHED PROCEDURES RECOMMENDED FOR TRIAL

THE STAINING OF ACID-FAST BACILLI IN PARAFFIN SECTIONS

G. L. Fite, Am. J. Path., **14**: 491. 1938

This note is to call attention to the above cited paper and to recommend a careful reading of it. The author does not set down his method in steps 1, 2, 3, etc., but discusses the subject in all its phases from the initial fixation to the mounting medium. A few of the recommendations made follow:

1. Alcohol is the fixation of choice though Zenker's, Orth's or Müller's fixed materials are satisfactory if the period of fixation is not prolonged and the tissues thoroughly washed in running water and the sections treated with permanganate and oxalic acid. Tissues fixed for a brief period in formaldehyde may be satisfactory but those preserved for long periods of time in this reagent are nearly useless for acid fast stains. The addition of 10 per cent formaldehyde to alcohol improves the fixation without affecting the staining of the bacilli.

For removing mercury deposits from the tissues Lugol's solution is satisfactory, but in removing the iodine both alcohol and sodium thiosulphate (in this order) are used. New fuchsin (fuchsin N B, magenta III) is recommended because of its greater solubility, and in the formula given below methyl alcohol is employed instead of ethyl, because of the greater solubility of the dye in the former.

Corbol Fuchsin (Fite):

New fuchsin	1 gram
Phenol crystals	5 grams
Methyl alcohol	10 cc.
Distilled water to make	100 cc.

Dissolve the dye in the mixture of phenol and alcohol and add the water slowly. Both the 10 per cent alcohol and the 5 per cent phenol are essential. Staining takes place more rapidly if the sections are treated in the following solution before staining:

Phenol crystals	5 grams
Alcohol	10 cc.
Water to make	100 cc.

To ensure maximum staining possible under the conditions of fixation the sections should be stained:

After alcohol fixation:

At 20°C	2–8 hours
At 37°C	1–4 hours
At 60°C	30 min. to 2 hours, or
At 90°C. (steaming)	5 minutes

After all other fixation:

At 20°C	16–24 hours
At 37°C	12–16 hours
At 60°C	8–12 hours, or
At 90°C	5 minutes

Of the three common methods commonly employed: (1) staining at room temperature; (2) staining immersed in the dye at elevated temperature, 37–70°C.; and (3) staining by steaming on the slide, the first is simplest and slowest. The second is most effective and most injurious to tissues. The great virtue of the third is its rapidity.

For a decolorizing agent 1–5 per cent hydrochloric acid in alcohol (70–95 per cent) is satisfactory. The lower strength acid has nearly the same decolorizing effect of the higher and is preferred.

If the amount of residual fuchsin is objectionable it may be removed by bleaching with potassium permanganate and oxalic acid or other substances. The author has used 5 per cent aqueous solution of potassium cyanide. Caution must be used as all these agents remove some of the stain in the bacilli.

For a counterstain the simplest probably is methylene blue in a 0.1–1 per cent solution rendered slightly alkaline with a few drops of ammonia, but there are many other dyes which will be found reasonably satisfactory.

In discussing the permanency of the stain the author states that he has learned to distrust the very brightly and brilliantly stained organisms as they are likely to fade for reasons unknown. If traces of alcohol remain in the section the bacilli may be expected to fade. Sections must not be allowed to stand in alcohol. An acid balsam or dammar will also bleach the organisms.

A NOTE ON THE STAINING OF TUBERCLE BACILLI IN SECTIONS

Dorothea S. Fuller, J. Lab. & Clin. Med., **23**: 416. 1938

Reagents:

1. Iron alum, 5 per cent aqueous solution.
2. Hematoxylin 1 gram dissolved in 80 cc. of hot, distilled water, cooled, and 10 cc. of glycerin and 10 cc. of 95 per cent alcohol added.
3. Saturated alcoholic solution of picric acid, two parts and 95 per cent alcohol, 1 part.
4. Saturated alcoholic solution of fuchsin 16 cc., aniline water 84 cc.
5. Nitric acid 3 per cent in 95 per cent alcohol.
6. Distilled water to which a few drops of ammonia have been added.
7. Light green (Grübler) 1 per cent aqueous solution.

Procedure:

1. Run slides down to water as usual, and rinse in distilled water.
2. Mordant in iron alum solution 5 minutes at 45 to 50°C.
3. Rinse in tap water.
4. Hematoxylin 5 minutes at 45–50°C.
5. Differentiate in the picric acid until only the nuclei are stained, 5 minutes or longer.
6. Wash in running water at least 15 minutes.
7. Pour on fuchsin solution and heat to steaming, but do not boil. Let cool for three minutes, heat again, and again allow to cool for three minutes.
8. Destain in the acid alcohol until the fuchsin starts coming off in clouds; rinse in tap water and continue with the acid alcohol until the sections are barely pink.
9. Rinse and put in the weak ammonia water for a few seconds.
10. Wash in running water for at least ten minutes.
11. Stain in the light green solution for five minutes.
12. Rinse in water, dehydrate quickly, clear in xylol, and mount as usual.

The advantages claimed by the author are that the method is suitable for formalin fixed tissues as well as Zenker's, it gives excellent nuclear and cytoplasmic details, excellent for photomicrography, and that the tubercle bacilli are stained equally well in all tissues including the brain and meninges.

CONCENTRATION OF TUBERCLE BACILLI FROM SPUTUM BY CHEMICAL FLOCCULATION METHODS

J. H. Banks, H. F. Clark, and Harry Feldman, J. Lab. & Clin. Med., **23**: 736. 1938

Reaents:

1. Digestor: 4 per cent sodium hydroxide which contains 0.2 per cent potassium alum and 0.002 per cent bromthymol blue.
2. Hydrochloric acid—approximately 2.5 N (25 per cent of conc. HCl by volume).
3. Ferric chloride solution (1 per cent of $FeCl_3$ in distilled water).

Procedure:

1. Mix 5 cc. of sputum with an equal volume of the digestor. Digest in water bath at 37°C. for thirty minutes with occasional shaking
2. Add 2.5 N HCl drop by drop with shaking until color of indicator denotes approximate neutrality. Shake for thirty seconds. If flocculation fails to occur in less than five minutes add 0.2 cc. of the ferric chloride solution and shake.
3. Centrifuge for five minutes at high speed and discard the supernatant fluid.
4. Prepare smears on slides, fix by heat, and stain by Ziehl-Neelson method.

It is claimed by the authors that a five minutes centrifugation of the alum treated sputum collects the bacilli more completely so that a unit of the sediment contains 3 to 7 times more bacilli than can be collected by direct centrifugation of the same specimen, and that the method does not interfere with the cultivation of the bacilli.

A COMBINED GRAM-METHYL GREEN-PYRONINE STAIN FOR FORMALDEHYDE TISSUE

James R. Lisa, Arch. Path., **26:** 728. 1938

Reagents:

1. Crystal violet, 1 gram is dissolved in 10 cc. of buffer phosphate solution of pH 6.6 to 7 and diluted to 100 cc. with distilled water.
2. Resublimed iodine, 2 grams, is added to 10 cc. of normal sodium hydroxide diluted to 30 cc. The iodine solution is shaken frequently and gradually diluted to 100 cc.
3. In a flask place 0.65 gram of Methyl Green NG 10 (National Amiline Company) and 0.1 gram of pyronin yellow special (National Aniline Company). Hot distilled water 100 cc. is added and the solution allowed to stand for three to four days. Store in a well stoppered amber glass bottle.

Technic:

1. Decerate and hydrate as usual.
2. Stain with crystal violet solution 5 minutes. Wash thoroughly.
3. Place in alkaline iodine solution 5 minutes. Wash.
4. Decolorize with pure acetone. Wash.
5. Stain with methyl green-pyronine solution for one to two minutes. Wash.
6. Decolorize with acetone, clear in two changes of xylene and mount in balsam.

The advantages claimed are that it combines the results obtained by Gram's method with the cellular differential features of the Unna-Poppenheim stain, that the dye solutions are stable and the method is rapid and simple.

ANNOTATIONS, MINOR CONTRIBUTIONS, QUERIES

YELLOW "ADDITION" FILTER FOR COLORIMETRY*

W. P. Stowe

In the article on the rose bengal test of liver function in the recent (second) edition of "Approved Laboratory Technic" edited by Kolmer and Boerner, reference is made to picric acid stained filters to compensate for the more yellow tint often found on one side of the colorimeter field. These filters are easily made by cutting cleaned X-Ray film into strips one inch wide and about six inches long. These are put into a Coplin staining jar about two-thirds full of saturated water solution of picric acid. At intervals a strip is pulled out, rinsed well in running cold water and blotted dry. Good intervals for a graded series are two, five, ten, twenty and sixty minutes, two, five, twelve and twenty-four hours.

Then with a sharp razor blade and a three by one inch microscope slide for a guide, the strips are trimmed so the line between stained and unstained portions is exactly in the centre. This three by one inch size fits the illuminated glass in the base of the Klett colorimeter, on which it is placed with the yellow end of the filter under the less yellow half of the field. By trying different filters and varying colorimeter settings a combination can be found where the colors exactly match. For the Duboscq type colorimeter larger filters should be made and held closely beneath the cups.

If the filters cloud up for a while, when freshly made, they can be cleared by rinsing in cold water and wiping with a soft cloth.

These filters are also useful in the phenolsulphonephthalein test, to balance the yellow of the urine specimen against the yellow-free standard, when a colorimeter is used.

AN IMPROVED METHOD FOR MAKING SMEARS FROM SPUTUM†

V. Rosalind Milligan

The method usually recommended for making smears from sputum is to press two slides together on particles of sputum. This procedure breaks up pus cells, distorts other structures, and there is danger of contaminating the fingers by the sputum which is frequently squeezed to the edges of the slides. Furthermore the thick film that is likely to result may dry slowly and stain unsatisfactorily. The method of spreading the sputum with the platinum loop without heat also gives a thick and not entirely satisfactory preparation.

The following method has been used for several months and obviates the

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† From the Clinical Laboratory of the Myers Clinic Hospital, Philippi, W. Va.

difficulties mentioned above. It has proved a rapid and satisfactory technique for making smears from sputum, especially in examining for tubercle bacilli.

This method is to pick up the selected portion of sputum on the platinum loop, place it on a slide which is passed over the flame and warmed sufficiently to start drying the sputum. The sputum is then spread over the slide with side to side and circular movements of the platinum loop and spread and warmed alternately until it is in a thin film and dry. The slide preparation is then ready for staining in the usual manner. Care must be taken to prevent burning the sputum. Rubbing the dried sputum should be avoided as this might discharge particles of dust into the air.

POTASSIUM TELLRUITE AND COPPER SULPHATE IN SABOURAUD'S MEDIUM FOR ISOLATION OF PATHOGENIC FUNGI

The addition of potassium tellurite (0.015 per cent) or copper sulphate (0.05 per cent) to Sabouraud's glucose agar prevents the growth of organisms other than fungi; moreover the per cent of positive cultures of fungi was markedly increased.

With the potassium tellurite medium the initial growth of the different species of fungi all became black in color. However, on microscopic examination no morphological changes were observed and on transplanting to plain Sabouraud's medium all the usual colonial appearances and color characteristics were resumed. T. L. Ch'in, Proc. Soc. Exper. Biol. & Med., **38**: 757, 1938.

CITRATE SOLUTIONS FOR PRESERVATION OF FLUID BLOOD

Because of the suspicion that some untoward reactions following citrate transfusions might be related to the citrate Joyce Cotter and W. J. MacNeal studied citrate solutions put out by different manufacturers, as well as those of their own making. A wide variation of pH values was found though all were high on the alkaline side up to 8.7. The solutions were tested by mixing 1 cc. of freshly drawn human blood with 4 cc. of the undiluted citrate solution and incubating at 37°C. for 20 hours, placed in the refrigerator for 24 hours and then left at room temperature for a week. There was marked hemolysis in those tubes with citrate solutions of pH 8.5, 8.2 and 8.1. In the citrate of pH 8.0 the hemolysis was distinctly less. When citrate solutions of pH 7.1 to 7.5 were used the blood remained unchanged for many days, even at room temperature. Citric acid 2.5 per cent in 0.6 per cent sodium chloride was used to bring the citrate solutions to the desired pH. Proc. Soc. Exper. Biol. & Med., **38**: 757, 1938.

MANNITOL FERMENTATION AS AN INDICATOR FOR CONJUNCTIVAL PATHOGENICITY OF STAPHYLOCOCCI

The author has simplified his test for determining the conjunctivitis-producing toxin of staphylococci by inoculating phenol red-mannitol agar (Difco)

plates directly from the conjunctiva and incubating over night. The reaction is considered positive if the medium around the colonies has changed from pink to yellow.

Of 92 strains which showed definite toxin production all but 1 fermented mannitol and of the 104 non-toxic strains only 4 showed fermentation, a correlation of 97.4 per cent. PHILLIPS THYGESON, Arch. Ophth., **20:** 274, 1938.

A NEW TEST FOR GLOBULIN IN THE CEREBRO-SPINAL FLUID

It is difficult to imagine a simpler test than Pandy's for globulin in the Cerebro-spinal fluid and yet K. O. Newman (Lancet **1:** 1333 (June 11, 1938)) describes a test which seems to have advantages.

His reagent is a 5 per cent pure tannic acid in distilled water. About 1 cc. is placed in a watch glass and one drop of cerebro-spinal fluid is allowed to run into it. A result similar to Pandy's is obtained but has the advantage that the precipitate remains constant for a considerable period, whereas in Pandy's test it vanishes after a few minutes. The author contrasts the ease of preparation of his reagent with the somewhat lengthy, if not difficult, preparation of a saturated aqueous solution of phenol. He states that the reagent keeps well. This has not been the experience of the reviewer, but if a 2 per cent phenol solution is used to make up the reagent instead of distilled water there should be no difficulty.

A NOTE ON THE CULTURE METHOD FOR ISOLATION OF TUBERCLE BACILLI FROM CONTAMINATED MATERIALS*

FRANK G. PETRIK

It has been brought to my attention that the sodium hydroxide technique as described in a previous paper[1] is not entirely clear. The following description is given to clarify some of the obscure points.

1. Mucoid sputa are mixed with an equal volume of 3 per cent sodium hydroxide and shaken in a shaking machine for thirty to forty-five minutes. They are then neutralized to phenolphthalein with 15 per cent (by volume) sulfuric acid. One extra drop of acid is added after the faint pink end point is reached so that the solution is just colorless. They are then centrifuged in a sterile tube covered with a sterile rubber cap.

2. Moderately muco-purulent and muco-purulent sputa are treated in a similar manner except that they are sometimes centrifuged after the faint pink end point is reached because further addition of acid would produce a larger amount of protein precipitate.

3. Purulent sputa are first diluted with an equal amount of sterile physiological salt solution then treated with sodium hydroxide as above and neu-

* From the Laboratory of the Homer Folks Tuberculosis Hospital, Oneonta, N. Y.

tralized. Some of these sputa cannot be fully neutralized without producing an excessive amount of sediment, therefore, they are only partially neutralized. Sulfuric acid is added with frequent shaking until the specimen is no longer gelatinous but appears watery when shaken. They are then centrifuged and one or two drops of sterile 3 per cent (by volume) hydrochloric acid is added to the sediment to neutralize the excess alkali. In a number of instances the sediment was first suspended in 5 cubic centimeters of physiological salt solution, neutralized and again centrifuged. The extra acid is added only to the sediments from these partially neutralized sputa. If extra acid were added to the sediment from neutralized sputa the reaction would be too acid for obtaining good growths of tubercle bacilli. Phenol red has been used for the past six months in place of phenolphthalein and found to be a more satisfactory indicator.

REFERENCE

(1) Petrik, Frank G.: Amer. Jour. Clin. Pathol., Technical Supplement, **2**: 134, 1938.